सत्यमेव जयते

INDIAN AGRICULTURAL

RESEARCH INSTITUTE, NEW DELHI

JOURNAL OF

AGRICULTURAL

RESEARCH

VOLUME 76

JANUARY 1–JUNE 15, 1948

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to V. R. Gardner, Director, Michigan Agricultural Experiment Station, East Lansing, Mich., who is chairman of the Station subcommittee.

CONTENTS

ERRATA AND AUTHOR'S EMENDATIONS

Page 234, second line from bottom, "furits" should be "fruits".

Page 239, paragraph 4, line 9, "pelargonium" should be "Pelargonium".

Page 255, line 2, "through" should be "though" and "bizzarria" should be "Bizzarria".

Page 258, line 6, "crosses" should be "crossed".

Page 322, paragraph 2, line 10, "favors" should be "factors".

INDEX

APPLE-TREE RESPONSE TO NITROGEN APPLIED AT DIFFERENT SEASONS [1]

By J. R. MAGNESS, *head horticulturist in charge*, L. P. BATJER, *senior pomologist*, and L. O. REGEIMBAL, *associate plant physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration* [2]

INTRODUCTION

At present nitrogen occupies a far more important place in the fertilization of apple (*Malus sylvestris* Mill.) orchards than any of the other nutrient elements. Ballard and Volck (*2*) [3] in 1912 were among the first to demonstrate experimentally that apple trees would respond favorably to applications of nitrogen. During the following decade results of numerous investigations showed conclusively the value of nitrogen in increasing growth and yield of apple trees. So marked were the responses obtained that the use of nitrogenous fertilizers for apples spread rapidly, and at present their use in some form is almost universal in commercial orchards. This increased use of nitrogen has made possible marked reduction in the amount of cultivation in apple orchards.

Formerly readily available forms of nitrogen were usually applied to apple orchards in the spring a few weeks prior to the bloom period. During the last 10 to 15 years in many fruit sections there has been an increasing tendency toward fall applications. This trend seems to date from the work of Hooker (*11*), who found that the nitrogen and the starch content of spurs were increased in the spring and early summer by applying nitrogen the preceding fall rather than in the spring of the current year.

Since the experiments of Hooker (*11*) much work has been done relative to the best time to apply nitrogenous fertilizers in orchards (*1, 14, 15, 16, 17*). The lack of agreement in the results obtained has led to differences of opinion among horticulturists as to the most desirable season to make applications. Recommendations for the eastern part of the United States vary from early-spring to fall applications; the latter are made from early September to mid-December. Split applications are also advocated, part of the nitrogen being applied in the spring and the remainder in the fall.

In the past many of the investigations relating to season of nitrogen applications were conducted with trees that were initially in a low

state of vigor, and responses were measured for only 1 or 2 years after treatment. Had such studies been of longer duration or had they been carried on with trees initially in a moderate or high level of vigor, it is possible that different conclusions might have been drawn. In commercial orchards moderately vigorous trees that receive annual applications of nitrogenous fertilizers are the rule rather than the exception. It was, therefore, the purpose of the present experiments to determine the effect on tree functions of annual applications of nitrogen made at different seasons of the year. The trees used over a period of several years were maintained at a moderately high level of vigor and in good production.

MATERIALS AND METHODS

The present studies were conducted in three rather widely separated apple orchards; two of these (the McDonald and the Dillon) were located in the Cumberland-Shenandoah fruit area and one at the Plant Industry Station, Beltsville, Md.

In the McDonald orchard near Charles Town, W. Va., 33-year-old York Imperial trees were used. These trees were growing in permanent bluegrass sod in Hagerstown clay loam. They had previously received yearly applications of nitrogenous fertilizer and were in good vigor. Hagerstown clay loam is considered an excellent soil for fruit trees. It is of residual origin, has an average field capacity of approximately 30 percent moisture, is well drained, and is of such physical make-up that it affords deep root penetration.

From the fall of 1937 through the 1941 season yearly applications of 10 pounds of $NaNO_3$ (sodium nitrate) per tree were made to five randomized trees per treatment at the following seasons:

1. Early fall (during September unless otherwise noted).
2. Late fall (November 15 to December 1, after the trees had shed their leaves).
3. Early spring (during April, approximately 3 weeks prior to bloom).
4. Late spring (during May, at the time of petal fall).
5. Midsummer (during the second half of July).

In addition, five trees received no nitrogen fertilizer after the grower's application in the spring of 1937. Fall treatments were begun in 1937 and spring and midsummer ones in 1938. Prior to treatment a sparse growth of sod beneath each tree was removed from the area of application. The nitrate of all applications was evenly distributed beneath each tree from the trunk out to approximately the outer spread of the branches. It approximated 45 p. p. m. (parts per million) of nitrate nitrogen in a foot of soil in the area covered.

In the Dillon orchard near Hancock, Md., 25-year-old Delicious trees were used. These trees were growing in a Dekalb shale loam, a shallow soil (usually not more than 2 to 2½ feet to unweathered shale) with a field capacity of approximately 20 percent moisture. Because of the limited depth of rooting on such soil fruit trees frequently lack sufficient moisture for optimum growth. Only 8 pounds of sodium nitrate per tree instead of 10 pounds was used on these smaller trees; this amount gave approximately the same concentration (45 p. p. m.) in the area to which it was applied. The experimental design was identical with that described for the York Imperial trees.

After four seasons of fertilizer treatment, two of the check trees in each orchard received nitrate in the spring of 1942 and five trees in each orchard that had received one of the spring applications were left untreated, to determine how rapidly change in the nitrogen levels of the trees would occur.

In the orchard at the Plant Industry Station, Beltsville, Md., trees 7 years old at the start of the experiment were used. They were growing in Sassafras gravelly loam of rather low fertility, maintained in a grass sod. Five trees of each of three varieties (York Imperial, Starking Delicious, and Rome Beauty) were included for each treatment. The treatments were on individual trees randomized throughout the orchard. The initial rate for a full application was 2½ pounds of sodium nitrate per tree. This amount was increased as the trees increased in size (see tables 7 and 8). The experiment included nine treatments. Five of these represented seasons of application similar to those in the McDonald and Dillon orchards. Two additional treatments consisted of nitrogen applied at the early-spring and late-fall dates, but in only half the amounts of the full treatments. Another treatment was of the full yearly amount, but split between two application dates (late-spring and early-fall); while the final treatment consisted of only half the full application, but also split between the two dates.

Since one of the chief objectives of these experiments was to determine nitrogen intake and utilization as related to season of application, samples for nitrogen analysis of blossoms, shoots, leaves, bark, wood, and roots were taken at intervals throughout the course of the experiments. These tissues were analyzed for total nitrogen by the official Kjeldahl-Gunning-Arnold method. In 2 of the orchards soil samples were taken at intervals with sampling tubes at 4 points under each tree, in order to follow the nitrate distribution at the various soil depths resulting from the different treatments. The phenoldisulfonic acid method was used, and the results were expressed in parts per million of nitrate nitrogen on the basis of air-dry soil. All sampling of soil, as well as of plant tissue, was done on an individual-tree basis. Leaf samples comprised 1 leaf midway of the shoot from each of 30 representative terminal shoots. Dormant and current shoots, as well as blossom clusters at appropriate sampling dates, were taken at 30 to 50 points throughout the tree. Root samples were taken in the surface foot of soil at 4 places beneath the spread of the branches. Unless otherwise stated, only the small roots less than about 6 mm. in diameter were selected for analysis.

Fruit-set records were obtained by counting 1.200 to 2,000 blossom clusters on 4 typical limbs in each tree. Two of these limbs were near the top of the tree and 2 in the lower half. Fruit set on these limbs was determined after the June drop. Color of fruit was determined at harvest by separating all of the fruit into 4 color grades based on the percentage of the total surface of good red color. The total average color for each tree was then calculated from the amount of fruit falling into each grade. Each year careful growth and bloom estimates were made on an individual-tree basis, and yield and fruit-size records were obtained. In the young orchard at Beltsville increase in trunk circumference was used as a measure of growth.

EXPERIMENTAL RESULTS IN McDONALD AND DILLON ORCHARDS

NITRATE-NITROGEN CONTENT OF SOIL

McDONALD ORCHARD

Root absorption, leaching, and biological fixation are the most common factors responsible for changes in the nitrate-nitrogen content of the soil after applications of sodium nitrate. Data for the nitrate nitrogen recovered at various depths in the McDonald orchard after the early-fall, the late-fall, the early-spring, the late-spring, and the midsummer applications are shown in figures 1 to 5, respectively.

As stated previously, the applications of sodium nitrate were calculated to give approximately 45 p. p. m. of nitrate nitrogen in a 1-foot depth of soil. From the data in figure 1 it is apparent that after early-fall applications most of the nitrate applied was recoverable even in the following early spring. Thus, almost as much nitrate was recovered on April 20, 1938, as had been applied on October 15, 1937. In 1939 the spring sampling was not made until May 11, approximately 2 weeks after full bloom. By that time the recoverable nitrate was approximately half the amount applied on September 2, 1938. Similarly, of the amount applied on September 2, 1939, approximately three-fourths was recovered on April 2, 1940, before the beginning of growth. The only exception to a relatively high recovery in the early spring was in 1941, when only approximately one-fourth as much nitrate was recovered on April 9 as had been applied the previous September. Rainfall had been unusually heavy after the date of application in the fall of 1940.

From October 15, 1937, to April 20, 1938, approximately 15 inches of rain fell. Most of the nitrate recovered on the later date was in
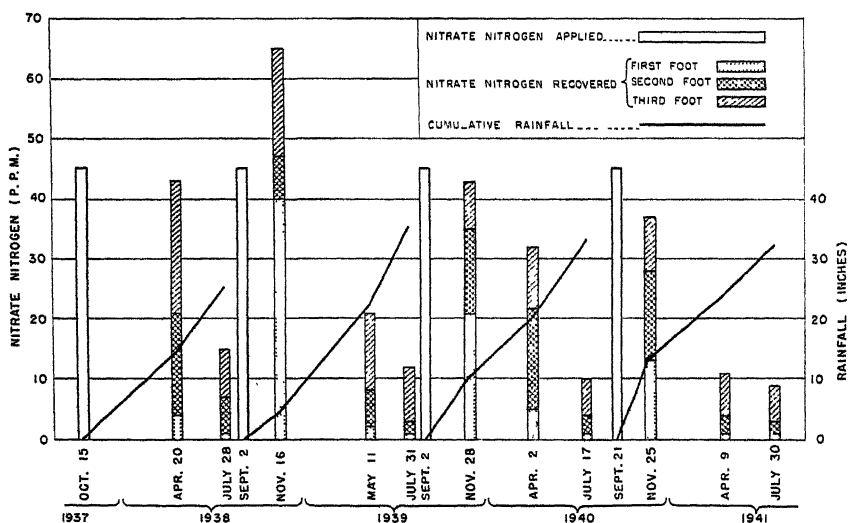
FIGURE 1.—Nitrate nitrogen applied annually in early fall and amounts recovered from the soil at various intervals thereafter, McDonald Orchard. Cumulative rainfall from date of application also indicated.
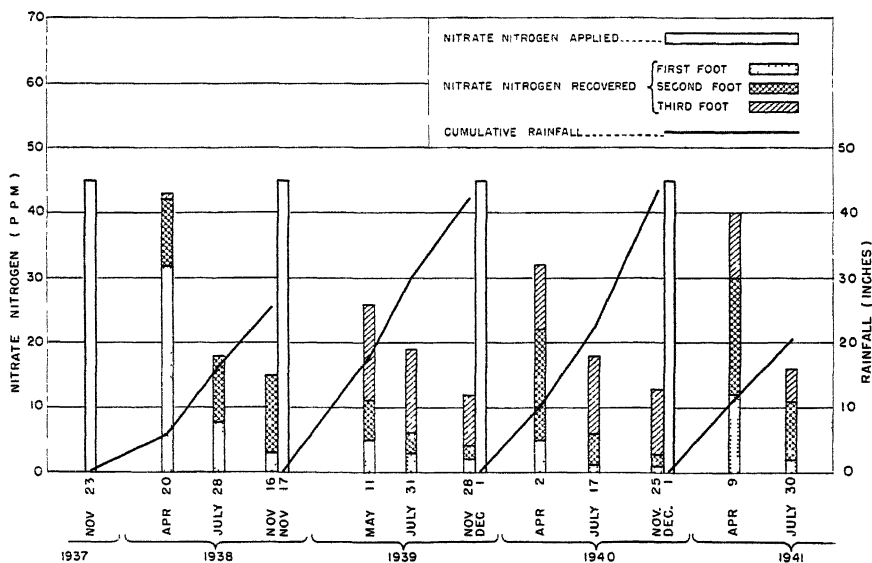
FIGURE 2.—Nitrate nitrogen applied annually in late fall and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.
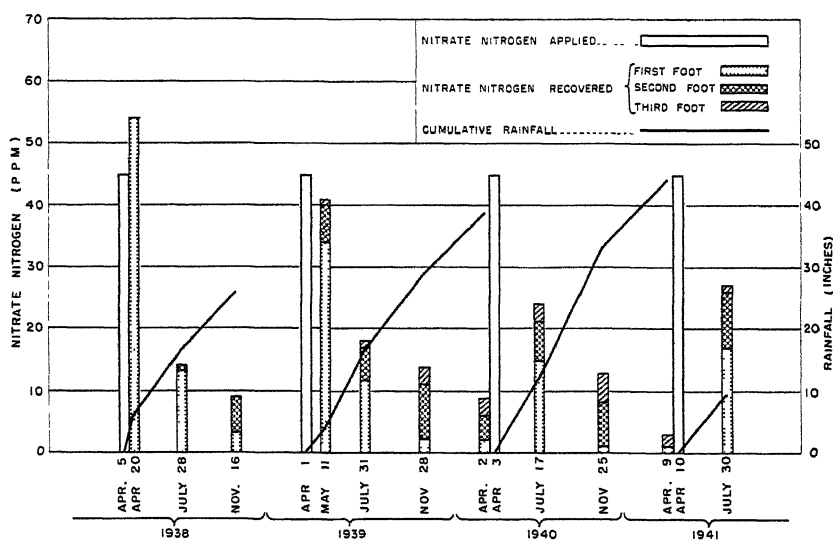
FIGURE 3.—Nitrate nitrogen applied annually in early spring and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.
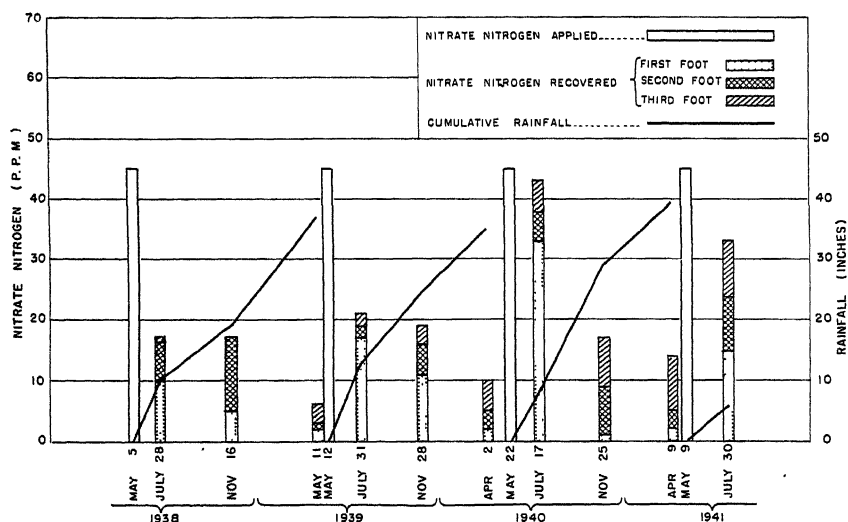
FIGURE 4.—Nitrate nitrogen applied annually in late spring and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.
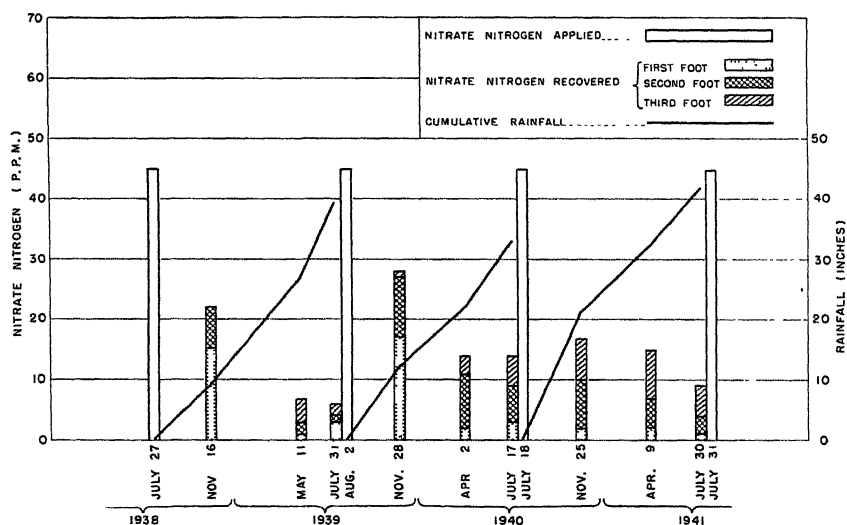
FIGURE 5.—Nitrate nitrogen applied annually in midsummer and amounts recovered from the soil at various intervals thereafter, McDonald orchard. Cumulative rainfall from date of application also indicated.

the second and third feet of soil. Between September 2, 1938, and May 11, 1939, there were 22.5 inches of rain. Most of the nitrate recovered on the later date was in the third foot of soil. From September 2, 1939, to April 2, 1940, 20 inches of rain fell, whereas from September 21, 1940, to April 9, 1941, the rainfall was just over 24 inches. It is possible that the heavier rainfall during the fall and winter of 1940–41 resulted in appreciable leaching of nitrate to below the 3-foot depth of sampling.

It is of interest to note that, although most of the nitrate that had been applied in the fall could be recovered in the following early spring, under the conditions of this test most of it was in the second- and third-foot depths. Relatively small quantities remained in the surface foot, where tree-root concentration is highest. However, in this orchard there was fairly good root distribution to a depth of at least 4 to 5 feet.

In general the pattern for nitrate recovery from late November applications was very similar to that from September applications (fig. 2). Most of the nitrate could be recovered the following spring and most of it was in the second and third feet of soil. The only exception occurred in the spring of 1938 when, after a very dry winter with only 6 inches of rainfall between November 23, 1937, and April 20, 1938, most of the nitrate was still in the top foot.

Figure 3 shows the nitrate recovery from early-spring applications. Shortly after the spring application, practically all of the nitrate could be recovered. By midsummer, represented by the late July samplings, on the average about half of the nitrate had disappeared. It is of interest to note, however, that even at the time of leaf fall in November, approximately one-fourth of the nitrate that had been applied in the spring could still be recovered. It seems probable that most of this disappearance of nitrate nitrogen was by root absorption. In these experiments there was little evidence of disappearance of nitrate nitrogen at seasons of the year when the trees were not in an active state of growth.

It is also of interest to note that at the time of the midsummer sampling (late July) most of the recoverable nitrate from early-spring applications was still in the top foot of soil even though 10 to 17 inches of rainfall had occurred. Apparently the trees dried out the soil between periods of rainfall, and there was relatively little movement of nitrate from the top foot into the lower soil depths under these conditions. Some was recovered in the second foot, but almost none in the third foot.

After the late-spring applications (May) there were relatively larger amounts of nitrate retained in the soil in July and in November than after the early-spring applications (fig. 4). There appeared to be relatively little disappearance between July and November. This relatively limited disappearance during late summer was evident also from the early-spring applications. If it is assumed that the disappearance was largely a result of absorption by the trees, the results would indicate that the first half of the growing season, or the period of rapid leaf expansion, was the period of most rapid absorption of nitrates from the soil.

When the fertilizer was applied in late July, approximately half of the nitrate had disappeared by late November (fig. 5). Thus, ab-

sorption during the late summer appeared to be relatively more active on trees that had not received applications in the spring. These trees were relatively low in nitrogen at the time of application, which was after terminal and leaf growth had been completed for the season. Under these conditions absorption appeared to be relatively greater during the late summer than in trees better supplied with nitrate as a result of the spring application.

<center>DILLON ORCHARD</center>

Because the Dillon orchard is growing on relatively shallow soil with undecomposed shale at a depth of 2 to 2½ feet, it was not possible to sample the soil below the 2-foot depth. The soil was somewhat lighter in texture with less water-holding capacity than that of the McDonald orchard. Since much of the nitrate applied in the McDonald orchard was carried below the 2-foot depth by the following spring, it may be presumed that much of the nitrate in the Dillon orchard was carried to a depth below that of possible sampling. Rainfall data were not available from any point near the Dillon orchard, but it may be presumed that the seasonal averages were approximately similar to those at the McDonald orchard, about 35 miles away.

The data in figures 6 to 10 for the various dates of application of nitrate nitrogen at the Dillon orchard bear out these probabilities. After early-fall applications generally less than one-fourth of the nitrate could be recovered the following spring. Much of that not recoverable may be presumed to have leached below the depth of possible sampling and possibly below the rooting depth. Data on nitrogen content of the roots presented later (table 1), however, indicate that during the dormant season some intake of nitrogen occurred in these trees.
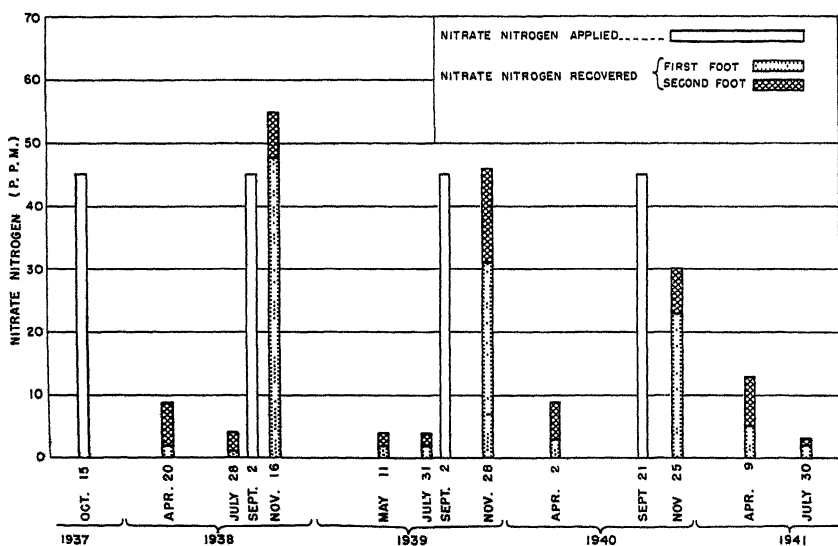
FIGURE 6.—Nitrate nitrogen applied annually in early fall and amounts recovered from soil at various intervals thereafter, Dillon orchard.
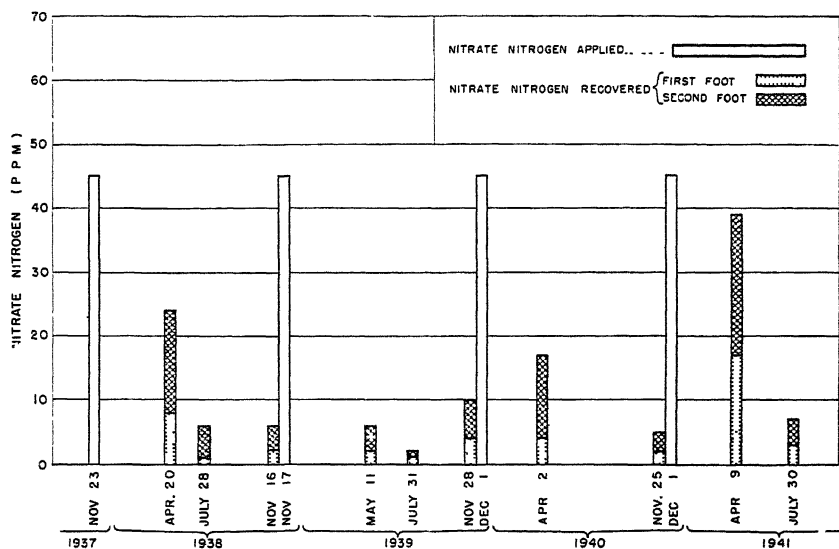
FIGURE 7.—Nitrate nitrogen applied annually in late fall and amounts recovered from soil at various intervals thereafter, Dillon orchard.
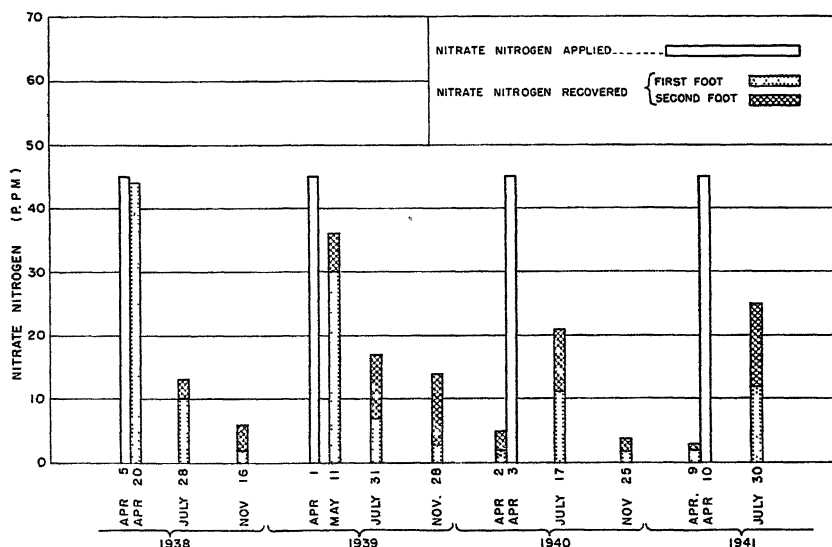
FIGURE 8.—Nitrate nitrogen applied annually in early spring and amounts recovered from soil at various intervals thereafter, Dillon orchard.
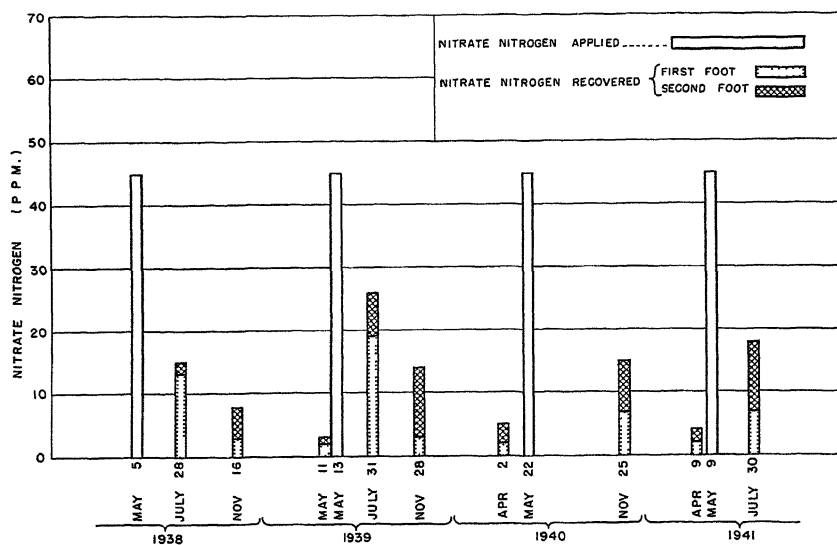
FIGURE 9.—Nitrate nitrogen applied annually in late spring and amounts recovered from soil at various intervals thereafter, Dillon orchard.
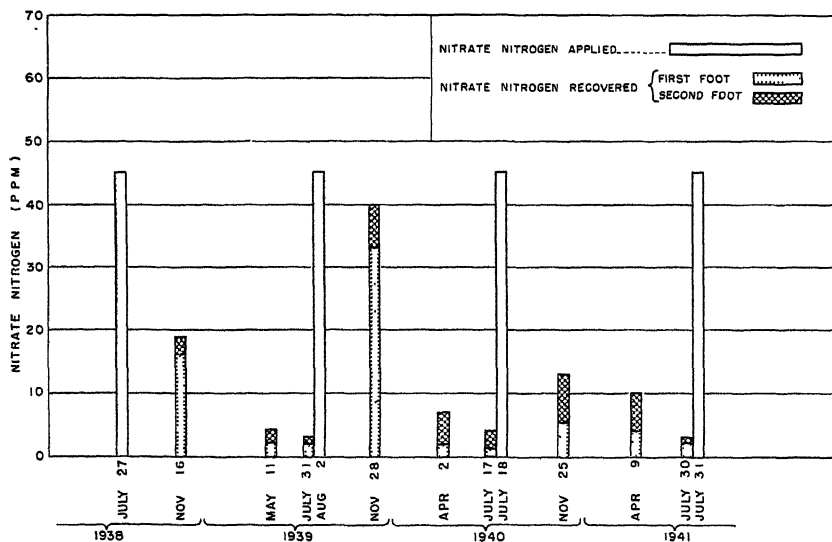
FIGURE 10.—Nitrate nitrogen applied annually in midsummer and amounts recovered from soil at various intervals thereafter, Dillon orchard.

For the early-spring applications the pattern is substantially similar to that of the McDonald orchard. On the average, approximately one-half of the nitrate could be recovered in late July and there was a rather marked further decrease between July and the end of the growing season. The results from the late-spring and midsummer applications also were substantially similar to those for the McDonald orchard.

Although there was little evidence of marked absorption of nitrate during the dormant season, the results on disappearance of nitrate in these two orchards would indicate that on a deep and fairly retentive soil with good root distribution there is little probability of loss of nitrates to the deep subsoil below the zone of root penetration when the nitrate is applied in the fall. On the other hand, where the depth of rooting is restricted by hardpan or rock or for other reasons, considerable of the nitrate applied in the fall may be carried below the active root zone before the following spring.

NITROGEN CONTENT OF VARIOUS PARTS OF TREES

ROOTS

Several investigators (*1, 4, 16, 17*) have reported the absorption of nitrogen by fruit-tree roots during the winter months when the tree tops are dormant. The data on Delicious apples (table 1) indicate significant increases in the nitrogen content of the roots during the dormant period that followed applications of nitrogen in the fall of 1937. Also in this experiment, during the dormant period of 1938–39 the nitrogen treatments in which the nitrogen content of roots was at a relatively low level at the beginning of the dormant period (early-spring, late-spring, and late-fall treatments) again showed appreciable increases prior to the beginning of growth the following spring. During this same period the midsummer and early-fall treatments, with a higher nitrogen content on December 1, 1938, showed no appreciable change in root nitrogen from that sampling date to March 15, 1939. The authors (*4*) previously showed that roots with a lower nitrogen content at the beginning of dormancy had a much greater rate of increase in nitrogen intake during the dormant period; the lower rate of increase in roots with the higher nitrogen content was attributed to the fact that these roots were nearer to a condition of nitrogen equilibrium when the tree entered the dormant period.

As evidenced by the data in table 1, only a small increase in nitrogen content of the roots of York Imperial trees took place during dormancy and the increase was as great in the check trees as in those receiving nitrate. In view of the recent work of Hoagland and Arnon (*10*), showing a close relation of oxygen supply of the rooting medium to nutrient absorption, the possibility of insufficient aeration in this heavier textured soil may have been a contributing factor. The work of the authors (*4*), which showed the nitrogen increase in the roots of dormant apple trees growing in pots in sandy loam soil to be more than double that of trees similarly grown in clay loam, would seem to lend support to this hypothesis.

The average nitrogen content of the roots for the various treatments immediately before the initiation of growth are presented in the last column of table 1. It may be seen that the early-spring application

TABLE 1.—*Relation of nitrogen content of roots to season of nitrogen application, 1937–41*

Variety and treatment	Nitrogen content [1] (dry-weight basis)						
	Nov. 20, 1937	Mar. 17, 1938	Dec. 1, 1938	Mar. 15, 1939	Apr. 2, 1940	Apr. 8, 1941	Average (March and April)
York Imperial (McDonald orchard).	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check	0.61	0.69	0 49	0 61	0 56	0 52	0.59
Early-fall	.61	.69	65	72	.73	.65	.70
Late-fall	.53	.68	.60	.71	.66	.79	.71
Early-spring			.54	.64	.62	.75	.67
Late-spring			.65	.70	.79	.63	.71
Midsummer			.74	.84	.86	.78	.83
Delicious (Dillon orchard).							
Check	.69	.84	52	57	.55	.56	.63
Early-fall	.85	1.13	1.13	1 17	1.26	1.18	1.18
Late-fall	.70	1.33	1.02	1.18	1.19	1.06	1.19
Early-spring			.80	1 05	.81	81	.89
Late-spring			95	1.14	.93	1 18	1.08
Midsummer			1 24	1.20	1.21	1.16	1.19

[1] Difference necessary for significance at 5-per cent point· 0.19.

to the Delicious trees resulted in a significantly lower nitrogen content of roots at that time than did the other nitrogen treatments; among these others there was no significant difference. The nitrogen content of roots of check trees, to which no nitrogen was applied throughout the course of the experiments, was significantly lower than that of those given the various treatments. In the York Imperial experiment, however, the roots of check trees did not contain significantly less nitrogen at the time the samples were taken than did the roots of trees receiving nitrogen except in the midsummer application; nevertheless, they always averaged considerably lower.

The data on root analysis as a whole indicate some increase in nitrogen content of the roots during the dormant season, particularly of those of the Delicious on well-aerated soil. They do, however, corroborate the results of the study of the nitrates in the soil, which indicated only a limited absorption of nitrogen in the McDonald orchard during the dormant season by trees previously well supplied with nitrogen.

DORMANT TERMINAL GROWTH

The data presented in table 2 show no consistent changes in nitrogen content of shoots during dormancy (comparison of November or December and March analyses). In both experiments the nitrogen level of dormant shoots of the fertilized trees averaged higher than that of shoots of the unfertilized trees. However, there was little or no difference in nitrogen content of shoots from the different treatments, except for those of the Delicious trees that received the midsummer application. The content of shoots of the Delicious that received this treatment was in general significantly higher than that of the shoots that received the other treatments.

BLOSSOMS

During 1938, 1939, and 1941 samples of blossoms were taken at the most advanced "balloon" stage, just before opening, to determine whether there were differences in the nitrogen content due to nitrogen

TABLE 2.—*Relation of nitrogen content of dormant terminal shoots to season of nitrogen application, 1937–41*

Variety and treatment	Nitrogen content [1] (dry-weight basis)						
	Nov. 20, 1937	Mar 17, 1938	Dec. 1, 1938	Mar. 15, 1939	Apr. 2, 1940	Apr. 8, 1941	Average (March and April)
York Imperial (McDonald orchard):	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check	0 91	0.95	0.91	0 90	0.90	0 87	0.90
Early-fall	.98	1.02	1 08	.99	1.03	1 02	1.01
Late-fall	.92	.98	1.00	1 02	.95	1.02	.99
Early-spring			1.03	.93	.98	.97	.96
Late-spring			1.07	1.05	1.05	.99	1.03
Midsummer			1.12	1.04	1.09	.99	1.04
Delicious (Dillon orchard):							
Check	85	.91	.93	.86	.90	.96	.91
Early-fall	94	.96	1.10	1.00	1.05	1.07	1.02
Late-fall	.95	1.02	1 08	1.05	1 01	1.05	1.03
Early-spring			1.06	1.05	1.05	.99	1.03
Late-spring			1.11	1.00	1.05	1.05	1.03
Midsummer			1.08	1.14	1.12	1.20	1.15

[1] Difference necessary for significance at 5-percent point: 0.10.

treatment (table 3). There was little difference in the York Imperial blossoms resulting from the treatments, the blossoms from the check trees being about as high in nitrogen as those from the trees receiving the various treatments. The midsummer application resulted in the highest nitrogen level in the blossoms. The Delicious blossoms from the check trees were generally lower in nitrogen than those from fertilized trees, the differences being generally significant in 1938 and 1939, but not in 1941. The nitrogen content was generally above 4 percent of the dry weight in the case of the Delicious and a little lower in the case of the York Imperial. Thus, at the balloon stage approximately one-fourth of the dry material of the blossoms is of nitrogenous compounds, since proteins average about 16 percent nitrogen.

TABLE 3.—*Relation of nitrogen content of blossoms and of current shoot growth to season of nitrogen application, 1938–41*

Variety and treatment	Nitrogen content [1] (dry-weight basis)							
	1938					1939		1941
	Current shoot growth				Blossoms (Apr. 19)	Current shoot growth (Apr. 25)	Blossoms (Apr. 25)	Blossoms (Apr. 25)
	Apr. 19	Apr. 26	May 4	May 23				
York Imperial (McDonald orchard):	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Check	3.75	2.99	2 52	1.93	3.61	3 91	3.44	3.10
Early-fall	3.77	3.13	2.76	2.04	3.71	3.95	3.46	3.11
Late-fall	3.82	3.12	2.53	2 00	3.92	3.92	3.48	3.15
Early-spring	3 82	3.19	2.66	2.02	3.80	3.94	3.44	3.16
Late-spring				2.07		3.96	3.41	3.10
Midsummer						3.91	3.71	3.24
Delicious (Dillon orchard):								
Check	4.25	3.66	2 95	2.48	3.92	4.85	4.16	3.59
Early-fall	4.58	4.04	3.53	2.76	4.12	5.10	4.41	3.73
Late-fall	4 58	4.07	3.55	2.73	4.26	5.02	4.36	3.69
Early-spring	4.47	3.94	3.34	2.89	4.16	5.03	4.40	3.65
Late-spring				2.72		5.02	4.37	3.55
Midsummer						5.13	4.33	3.70

[1] Difference necessary for significance at 5-percent point: Shoot growth, 0.06; blossoms, 0.20.

There were marked differences in the nitrogen content of the blossoms in the different seasons. In 1941 the nitrogen content of the blossoms in every treatment was markedly below the corresponding 1939 levels. In the spring of 1939 there were no unusually warm days prior to the date of sampling, April 25. Of the previous 10 days, only 3 had had maximum temperatures as high as 70° F. Throughout late March and April, however, temperatures were sufficiently high to permit a rather steady development of the blossoms. In March 1941, on the other hand, practically no days were sufficiently warm to cause growth and little growth occurred until after the first week in April. Beginning with April 11, the maximum temperatures were above 70° every day through April 21, and on eight of these days they were above 80°. Thus, the blossoms and new shoot growth developed with extreme rapidity during the 2 weeks prior to sampling. It seems probable that this very rapid development was mainly responsible for the lower nitrogen content in the succulent blossom tissues. The translocation of nitrogen may have been relatively less affected by the higher temperatures than was the growth rate.

The number of fruit set per 100 blossoming spurs of both the Delicious and the York Imperial was actually higher in 1941, when the nitrogen content was low, than in 1939, when the nitrogen content was much higher. Some low-temperature injury had occurred prior to blossoming in 1939, but it did not appear to be extensive. In both years conditions for pollination were very favorable. Thus, under the conditions of this test the lower nitrogen content of the blossoms was not unfavorable for fruit set. Furthermore, throughout the full course of the experiment the fruit set on the check trees averaged as high as that on the nitrogen-treated trees (see table 6).

CURRENT SHOOT GROWTH

In the spring of 1938 current shoot growth was sampled four times during terminal elongation (table 3). On April 19, soon after the beginning of growth, the shoots (about half an inch long) on check trees had a slightly lower nitrogen content in most cases than those on trees treated at various seasons. The content of the shoots on the Delicious trees treated in early spring was lower than that of those treated at other seasons; otherwise season of treatment apparently had little effect. At the time of terminal-bud formation (about May 23), however, shoots from the Delicious trees treated in early spring had the highest nitrogen content. On this date and also at blossoming time in 1939 shoots from York Imperial trees that had received various treatments showed only slight differences, although all fertilized trees were significantly higher than the checks. In 1939 shoots from treated Delicious trees all had a higher nitrogen content than those from check trees, and those treated in midsummer and early fall had a significantly higher content than those fertilized at other seasons.

The data on current shoot growth shown in table 3 indicate also the rapid absorption and translocation of nitrogen resulting from spring applications. In 1938, after nitrogen applications on April 5 (early spring) and May 6 (late spring), the nitrogen content of current shoot growth of both varieties showed a significant increase when compared with check trees within 14 and 17 days, respectively. The

nature of the experiment made it impossible to check this result in other years.

The shoots all showed a rapid decline in nitrogen from the initiation of new growth until the end of the sampling period. On the average terminal growth ceased somewhat earlier on the check trees than on the fertilized ones. All trees had ample moisture when shoot elongation ceased. These data indicate that the time of cessation of terminal growth in mature apple trees is largely determined by the depletion of the nitrogen available for rapid transfer to the new developing tissues—assuming no other nutrient element to be limiting. The amount of the new terminal growth appears to be determined by the reserve nitrogen in the tree and the intake during the season of rapid development of new tissues.

FOLIAGE

Numerous investigators (*3, 7, 8*) have shown the nitrogen level of the leaves to be a very sensitive index to the nitrogen supply in the rooting medium and have generally reported a very close relation between the nitrogen content of foliage and the growth and vigor of fruit trees. The close relation between nitrogen content of foliage and leaf assimilation per unit area has also been established (*3, 9*).

The nitrogen content of leaves taken from the median parts of terminal shoots at intervals throughout the experiment is shown in table 4. In many instances differences statistically significant were obtained between certain treatments in a particular year, whereas in another year at approximately the same date there seemed to be either no difference or the difference was even in the opposite direction in certain cases.

Throughout the experiment there was no consistently significant difference between the nitrogen content of the foliage after early-fall, late-fall, or early-spring treatments. During the growing season the foliage from check trees was consistently lower in nitrogen than that from trees receiving nitrogen applications.

It might be expected that the late-spring application, made after most of the leaf system was expanded and too late to influence greatly the growth for the season, would result in a higher level of nitrogen in the foliage throughout the summer than would the dormant-season treatment. This was generally true of the York Imperial but not of the Delicious. The over-all average in June of nitrogen in the foliage of the Delicious variety resulting from the late-spring application was below that resulting from application in the fall or early spring. No explanation of these results is apparent.

It would be expected that for samples taken in early summer the midsummer applications would result in a somewhat lower nitrogen content of the leaves than would dormant-season or late-spring applications. This was true of the York Imperial, as shown by the average for the June samples. It was also true of the Delicious except that there was no difference between the late-spring and midsummer appplications.

During September the nitrogen content of the foliage of all trees that received nitrogen was essentially the same irrespective of the season of application (table 4). Heinicke (*9*) emphasized the importance of making a late-summer application of nitrogen in order to

TABLE 4.—*Relation of nitrogen content of foliage to season of nitrogen application, 1938–41*

Variety and treatment	Nitrogen content [1] of terminal leaves (dry-weight basis)													
	1938					1939			1940		1941		Average	
	May 24	June 22	Aug. 1	Sept. 13	Nov. 2	June 15	Aug. 1	Sept. 1	June 11	Sept. 19	June 17	Sept. 10	June	Sept.
York Imperial (McDonald orchard).	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per cent
Check	1.99	2 08	1.93	1 78	0 89	1.79	1 81	1 81	2 02	1.65	1 94	1 64	1 96	1.72
Early-fall	2.21	2.43	2 17	1 95	92	2 12	2 12	2.04	2.17	1.82	2.27	1 92	2 25	1.93
Late-fall	2.14	2 37	2 13	1.94	89	2 13	2.09	2 06	2.29	1 83	2 30	1 93	2.27	1.94
Early-spring	2 10	2.35	2 19	1 97	.87	2 17	2 14	2.05	2.28	1.87	2 27	1 94	2 27	1.96
Late-spring	2.17	2 46	2.23	2.03	95	2.18	2 15	2 01	2.48	1.88	2.31	1 80	2 36	1.93
Midsummer			2.04	2.01	.90	2 15	2.11	2.06	2.18	1.91	2 20	1 86	2.18	1.96
Delicious (Dillon orchard).														
Check	1.99	2 07	1 87	1 62	1.21	1 81	1.78	1.65	1.82	1 68	2.14	1.88	1.96	1 71
Early-fall	2 33	2 39	2.13	1.89	1.18	2.48	2.22	2.03	2.46	2 10	2 42	2 03	2 44	2 01
Late-fall	2.27	2.47	2 19	1.95	1.17	2.45	2.23	2 03	2.43	2 06	2 44	2 07	2 45	2 03
Early-spring	2 38	2.52	2.16	1.94	1 14	2 40	2.23	2.09	2 49	2.14	2 47	2 13	2.47	2.07
Late-spring	2.27	2.43	2 22	1 98	1.10	2 25	2.22	2 12	2.49	2.10	2 31	1.98	2.37	2 04
Midsummer			1.93	1.83	1.12	2.28	2 10	2.02	2 43	2 20	2 37	2.11	2.36	2.04

[1] Difference necessary for significance at 5-percent point· 0.05.

maintain or increase the dark-green color of apple foliage and so increase its efficiency in assimilation during the late summer and fall. With nitrogen-deficient trees such a practice would probably be beneficial and would doubtless accomplish the desired purpose. However, with trees that received annual application of nitrogenous fertilizers no difference was found in nitrogen content of foliage or in leaf color during the fall months as a result of differences in season of application. Since the close relation between nitrogen content and color and efficiency of foliage has been demonstrated (*3, 6*), there is little likelihood that any real difference in foliage assimilation that could be attributed to season of application occurred during late summer and fall in these experiments.

In 1938 the leaves were sampled at rather frequent intervals from late May until leaf fall. It is particularly interesting to note that samples of both varieties taken May 24 were consistently lower in nitrogen than those taken June 22. These results suggest that the leaves reach a low level of nitrogen content in the spring at about the time of cessation of terminal growth and that subsequently there is some increase in nitrogen in the foliage. Samples taken in midsummer (August 1) were generally slightly lower in nitrogen than those taken in June. The trend of nitrogen content was downward from June until September.

One set of analyses, the November 2 sampling, were made on leaves that fell when the branches were jarred. Approximately half of the foliage had dropped when the samples were collected. Branches were jarred and leaves that fell were taken and analyzed. In the York Imperial the nitrogen content of leaves was less than half the midsummer level, whereas in the Delicious it was slightly more than half. Thus, although much of the nitrogen apparently moved out of the leaves prior to leaf fall, a substantial portion was still present at that time.

In general, the leaf analyses indicate no significant differences in the levels of nitrogen maintained in these trees as a result of varying the time of application of sodium nitrate. Even in the Delicious, for which the data on nitrate in the soil indicated some loss of nitrates from leaching after the fall applications, this loss was not reflected in the nitrogen level maintained in the leaves. The nitrogen level resulting from the fall applications was as high as that from spring and midsummer applications.

VARIOUS TISSUES OF DORMANT TREES AFTER 4 YEARS OF TREATMENT

Much of the nitrogen contained in the various tissues of the tree is available for retransfer and reutilization. After 4 years of treatment it seemed desirable to determine to what extent the amount of reserve nitrogen contained in the different parts of the tree had been influenced by season of nitrogen application. Trees receiving the late-fall and early-spring treatments were selected for detailed analysis, since in many fruit districts it is at these seasons that nitrogenous fertilizers are most frequently applied.

The data for all the various tissues analyzed for nitrogen content do not show any differences of significance attributable to season of application (table 5). With some tissues certain numerical differences are apparent, but such differences are not consistent enough or of sufficient magnitude to be definitely related to season of application.

It will be noted from the data in table 5 that the various tissues of the unfertilized trees generally contain less nitrogen than those of the trees receiving nitrogen, the differences being greatest in the bark of limbs and the root tissues. By careful analyses Magness and Regeimbal (*13*) estimated a necessary yearly intake of about 1.5 pounds of nitrogen to maintain a mature apple tree in good production. From the nitrogen values contained in table 5 and the dry weights of the

TABLE 5.—*Relation of nitrogen content of various tissues of dormant trees after 4 years of treatment to season of nitrogen application, December 1, 1941*

	Nitrogen content (dry-weight basis)												
	Tops										Roots		
	Small limbs¹			Medium limbs²			Large limbs³				Less than 5-mm. diameter	Large roots	
Variety and treatment	Shoots	Bark	Wood	Bark	1-year-old sapwood	Old sapwood	Bark	1-year-old sapwood	Old sapwood	Heartwood		Bark	Wood
York Imperial (McDonald orchard):	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Check	0.937	0.830	0.245	0.609	0.348	0.134	0.623	0.302	0.118	0.087	0.562	0.427	0.187
Late-fall	1.028	.963	.301	.650	.375	.134	684	.328	.121	.090	.579	.418	.300
Early-spring	1.039	.941	.258	.665	.355	.131	.688	.291	.121	.088	.638	.488	.251
Delicious (Dillon orchard):													
Check	.912	.881	.246	.700	.260	.136	.671	.251	.137	.102	.585	.459	.342
Late-fall	1.110	.897	.250	743	.289	.150	.720	292	.146	.103	1.024	.608	.416
Early-spring	1.133	.950	.273	.754	.256	.144	.764	.260	.146	.109	.958	.587	.537
Difference necessary for significance at 5-percent point	116	.077	.044	.043	.044	.012	.054	.044	.008	.011	.241	.160	.204

¹ ½- to ¾-inch diameter.   ² 1- to 2-inch diameter.   ³ Over 3-inch diameter.

various tissues of an apple tree, it was estimated that the nitrogen-fertilized trees contained 0.6 to 0.7 of a pound more residual, or reserve, nitrogen than the unfertilized ones. This amount of nitrogen, which might be regarded as available for retransfer and reutilization, would be sufficient to meet a substantial proportion of the requirement of a tree for 1 year.

SET, COLOR, AND YIELD OF FRUIT

The data presented in table 6 show considerable consistency in fruit set of trees treated at different seasons. Average fruit set for the 4-year period was essentially the same regardless of season of fertilization or lack of fertilization. The relation of fruit color to nitrogen supply and intake has been shown previously (*5, 12*). It is interesting to note that in the present experiments little if any differences in fruit color were associated with season of nitrogen application. The much higher color of fruit from trees not receiving nitrogen is clearly shown in table 6. Although yield records on such a limited number of trees

TABLE 6.—*Relation of fruit set, color, and yield to season of nitrogen application, 1938–41*

Variety and treatment	Fruits per 100 blossoming spurs	Color	Yield	Variety and treatment	Fruits per 100 blossoming spurs	Color	Yield
York Imperial (McDonald orchard):	*Number*	*Percent*	*Bushels*	Delicious (Dillon orchard)—Continued	*Number*	*Percent*	*Bushels*
Check	44	58	19 6	Early-fall	35	49	10. 6
Early-fall	39	45	19.4	Late-fall	36	49	11. 6
Late-fall	41	41	19 2	Early-spring	30	47	10. 8
Early-spring	44	42	21. 4	Late-spring	35	50	12. 0
Late-spring	37	43	20. 4	Midsummer	31	53	10. 6
Midsummer	40	42	21. 6	Difference necessary for significance at 5-percent point	9	5	4.
Delicious (Dillon orchard):							
Check	32	66	8. 0				

(five per treatment) cannot be regarded as very reliable, the data do not suggest any great differences in fruiting performance. The amount of bloom per tree and the percentage set were recorded each year and careful growth estimates (terminal growth and density and size of foliage) were made at intervals throughout each growing season. No significant differences that could be attributed to season of nitrogen application were ever observed.

Withholding nitrogen from the check trees resulted in reduced growth and relatively small, light-green leaves the first season of these experiments (1938), and these manifestations became progressively more pronounced in 1939, with the trees exhibiting typical symptoms of nitrogen deficiency during that year. The growth of these trees, however, was not greatly different in 1940 and 1941 from that in 1939. The lower average yield of the check trees in the Delicious experiment reflects this reduced growth, and in the York Imperial experiment it was obvious that the yield of the check trees would ultimately be reduced if nitrogen applications were not resumed. Of interest is the fact that throughout these experiments nitrogen never became sufficiently limited to reduce fruit set of the check trees, though these trees quickly reflected the lack of adequate nitrogen for normal vigor and growth.

RATE OF RESPONSE TO NITROGEN FERTILIZER

In the spring of 1942 nitrogen was applied to two check trees and withheld from five trees formerly receiving spring applications in each of the two experiments. In September of that year in the York Imperial experiment the foliage of the two former check trees that received nitrogen in the spring contained 1.99 percent of nitrogen, whereas the foliage of the three remaining checks that did not receive nitrogen contained 1.64 percent. The average fruit color on the nonfertilized trees was much superior to that on the fertilized ones. When nitrogen was withheld in 1942 from five of the trees formerly receiving spring applications, the nitrogen content of the foliage was 1.71 percent in September, while that of the regularly treated trees was 1.96 percent. Fruit color averaged 50 percent and 41 percent, respectively. When the same procedure was followed in the Delicious experiment in 1942, the results were very similar. Thus, in these experiments, when nitrogen was applied in early spring to trees in moderately low vigor, its effect was clearly reflected in nitrogen content of foliage and in fruit color during the fall of the same year. Also, withholding nitrogen from trees in high vigor resulted in a sharp decrease in nitrogen content of foliage and in improved fruit color within a single season.

EXPERIMENTAL RESULTS IN THE PLANT INDUSTRY STATION ORCHARD

NITROGEN CONTENT OF FOLIAGE AND TREE GROWTH

The results pertaining to season of nitrogen application in the McDonald and Dillon orchards were obtained with mature apple trees. In order to determine the response of young trees to season of application, experiments were conducted over a 5-year period (1939–43) with Rome Beauty, Starking Delicious, and York Imperial trees between their seventh and eleventh years of age. Details of the experimental set-up are presented under Materials and Methods.

That various treatments resulted in considerable difference in growth (as measured by percentage of increase in trunk circumference) and in nitrogen content of foliage is shown in table 7. As would be expected, trees receiving half the full application of nitrogen (Nos. 7, 8, 9) contained less nitrogen in the foliage during late summer and also made a lower rate of growth than those receiving high-nitrogen treatments. The three treatments in which the trees had the highest nitrogen content of foliage and the greatest trunk growth were those that received the late-spring, midsummer, and split (spring and fall) treatments in the high-nitrogen series. No significant differences in growth rate or nitrogen content of foliage resulted from early-spring, early-fall, and late-fall applications at the high level or from late-fall and early-spring applications at the low level. The close relation between nitrogen content of foliage and trunk growth of trees shown in table 7 is graphically illustrated in figure 11.

NITROGEN CONTENT OF FOLIAGE AND FRUIT COLOR

The relation of nitrogen content of foliage and fruit color of Rome Beauty trees to season of nitrogen application is shown in table 8. In the high-nitrogen series development of fruit color and nitrogen
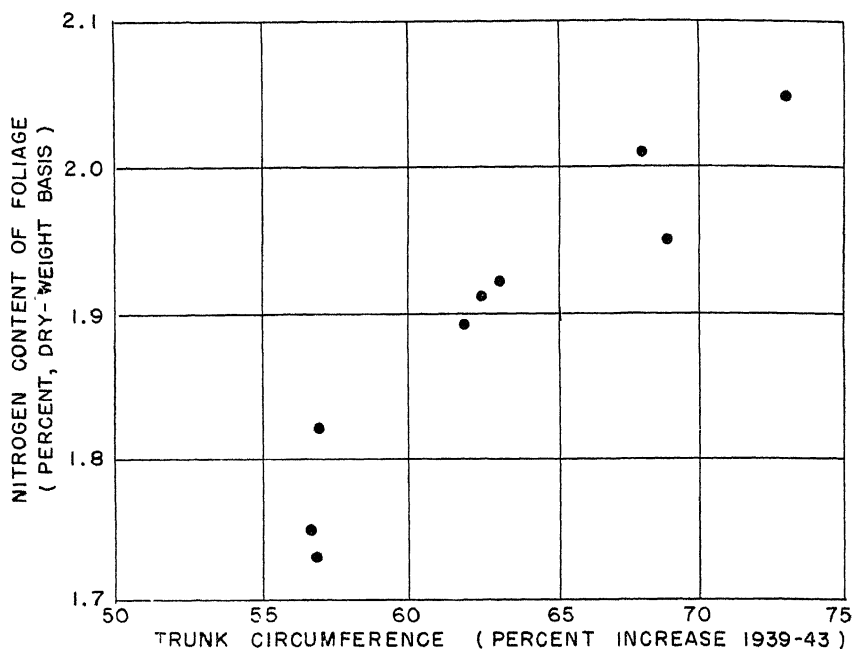
FIGURE 11.—Relation of average nitrogen content of foliage and percentage of increase in trunk circumference of York Imperial, Starking Delicious, and Rome Beauty apple trees 7 to 11 years of age.

content of foliage were essentially the same when treatments were made in early spring, early fall, and late fall; in the low-nitrogen series they were about the same when the treatments were made in early spring and late fall. With respect to the other treatments the data generally show that the treatments resulting in the highest nitrogen level of foliage and the greatest percentage of increase in growth (table 7) produced fruit with the least red color (treatments 2 and 3).

TABLE 7.—*Relation of nitrogen content of foliage and tree growth of York Imperial, Starking Delicious, and Rome Beauty apple trees to season of nitrogen application, 1939–43*

Relative rate of nitrogen application and treatment No. and description	Average increase in trunk circumference	Average nitrogen content of foliage [1]	Relative rate of nitrogen application and treatment No. and description	Average increase in trunk circumference	Average nitrogen content of foliage [1]
High.[2]	*Percent*	*Percent*	Low:[3]	*Percent*	*Percent*
1. Early-spring	62.5	1.91	7. Early-spring	56 9	1.73
2. Late-spring	73.0	2.05	8. Late-fall	56.8	1.75
3. Midsummer	68.9	1 95	9. Split (half late-spring, half early-fall)	56.9	1.82
4. Early-fall	63.3	1.92	Difference necessary for significance at 5-percent point	6.6	.05
5. Late-fall	61.9	1.89			
6. Split (half late-spring, half early-fall)	67.9	2.01			

[1] Leaves from terminal growth taken for analysis in late July or early August.
[2] Yearly applications of actual nitrogen (pounds per tree) were as follows: 1939, 0.40; 1940, 0.40; 1941, 0.48; 1942, 0.48; 1943, 0.52.
[3] Yearly applications of nitrogen (pounds per tree) were half the amounts applied in the high series.

TABLE 8.—*Relation of nitrogen content of foliage and fruit color of Rome Beauty apple trees to season of nitrogen application, 1939–43*

Relative rate of nitrogen application and treatment No. and description	Average nitrogen content of foliage [1]	Average color	Relative rate of nitrogen application and treatment No. and description	Average nitrogen content of foliage [1]	Average color
High: [2]	*Percent*	*Percent*	Low: [3]	*Percent*	*Percent*
1. Early-spring	1 87	56	7. Early-spring	1.69	66
2. Late-spring	2 04	43	8. Late-fall	1 73	63
3. Midsummer	1.91	39	9. Split (half late-spring,		
4. Early-fall	1.89	52	half early-fall)	1.75	56
5. Late-fall	1.85	52	Difference necessary		
6. Split (half late-spring,			for significance at		
half early-fall)	1 97	47	5-percent point	.09	6.6

[1] Leaves from terminal growth taken for analysis in late July or early August
[2] Yearly applications of actual nitrogen (pounds per tree) were as follows. 1939, 0.40; 1940, 0.40; 1941, 0.48; 1942, 0.48; 1943, 0.53.
[3] Yearly applications of nitrogen (pounds per tree) were half the amounts applied in the high series.

Furthermore, the highest fruit color occurred in treatments with low-nitrogen levels and low rates of growth (treatments 7 and 8). This relation of nitrogen content of foliage to color development in the fruit is shown graphically in figure 12. Reference to this chart shows
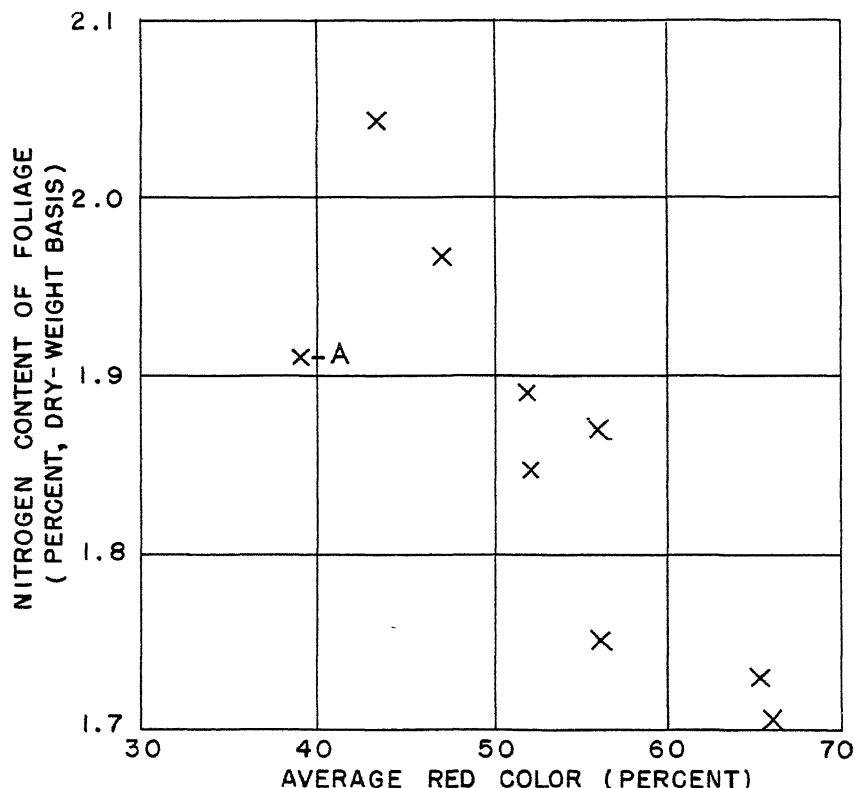
FIGURE 12.—Relation of average nitrogen content of foliage and average percentage of red color of fruit of Rome Beauty apples on young trees. x–A, Value for midsummer application.

approximately the same negative relation between nitrogen content of foliage and development of fruit color that was previously reported by the authors (*12*). With the midsummer treatment there was a much lower color of fruit than might have been expected from the nitrogen content of the foliage (fig. 12, x–A). An explanation of this variation is the fact that the yearly applications of nitrogen in this treatment were usually made at the time of the sampling of foliage for nitrogen analysis or immediately afterward. These young trees apparently absorbed and translocated nitrogen rapidly, and the nitrogen content of the foliage at the time of fruit coloring was relatively higher than at the sampling date. Thus, color development was poorer than would be expected from the nitrogen content of the foliage sampled (late July or early August).

DISCUSSION

In these studies nitrogen applications to mature apple trees made at five different times during the year, from early spring to late fall, failed to result in any essential differences in tree performance over a period of 4 years as measured by set, color, and yield of fruit and by general vigor of tree. The relation of nitrogen content of various tissues to tree vigor has been shown previously (*3, 15, 17*) and is rather generally recognized. Throughout these experiments extensive nitrogen analyses were made of blossoms, shoots, leaves, bark, wood, and roots of trees receiving the nitrogen at different seasons. In general, the nitrogen content of these tissues does not indicate any large or consistent differences due to time of nitrogen application. At certain times of year, however, significant differences in nitrogen content of some tissues were observed; these seemed to be related to season of nitrogen application. Thus, the data in table 1 indicate that nitrogen content of the roots in late March or early April was lower when nitrogen was applied in early spring than when it was applied at other seasons.

The root samples were taken just before the nitrate was applied in the early-spring treatment. In all other treatments the nitrate content of the soil was higher during the preceding months. This fact probably accounts for the slightly lower nitrogen content of the roots at the spring sampling. The nitrogen content of blossoms, young shoots, and leaves of trees receiving the early-spring treatment compared favorably with the content following treatments at other seasons. This indicates that in these vigorous trees, the nitrogen applied in early spring was in part taken into the tree within a short time after application.

The midsummer applications resulted in a relatively high level of nitrogen in roots and shoots during the dormant season. This application was made after the season's terminal growth was completed and could be expected to result in an increase in nitrogen content of the various tissues during the following dormant season. Nitrate nitrogen in the soil was at a low level in this treatment during the first part of the growing season. The nitrogen content of the foliage in June was lower than that for other times of application. In late summer, however, the nitrogen content of foliage was substantially the same for all application dates.

The examples just given serve to illustrate certain apparent trends

in nitrogen content of tissues as related to season of application. However, when the data as a whole are considered, it is rather obvious that under the conditions of these experiments different seasons of nitrogen application over a period of 4 years failed to influence materially the nitrogen level of mature apple trees. These results strongly suggest that, if the level of nitrogen metabolism is satisfactorily maintained within the tree, it makes little or no difference at what season of the year the soil supply is replenished.

These results were obtained with trees that had already received yearly applications of nitrogen prior to the initiation of the experiments. The rate of nitrogen application during the experiments was slightly in excess of the usual commercial rate for such trees. During the 4 years of the experiment the check trees, with no added nitrogen, yielded almost as well as the fertilized trees, although all their tissues showed a lower level of nitrogen and shoot growth was reduced. Fruit color on these trees was very much better than on those receiving nitrogen fertilizer. The soil in which the trees were growing is inherently fertile. A considerable amount of organic debris from old sod and fallen leaves had accumulated under the trees, and the check trees undoubtedly obtained considerable nitrogen from these sources. The superior quality of the fruit produced on these trees emphasizes the need for a critical evaluation of the effect of different levels of nitrogen on yield and quality of fruit on mature trees. The level of nitrogen appears much more important than the time of application in determining tree response.

Results of these studies with mature apple trees are at variance with those of a number of workers who reported considerable differences in tree response to season of nitrogen application (*1, 11, 15, 16, 17*). As pointed out earlier, in most instances such differences were obtained for a short period after applications of nitrogen to trees in moderate vigor or in most cases in extremely low vigor.

Results with young trees in these studies showed a very close relation between nitrogen content of foliage and growth of tree. In certain seasons of application the results with young trees are in close agreement with those obtained with mature trees. Thus, no significant difference was obtained in nitrogen content of foliage or in growth, when nitrogen was applied in early spring, early fall, or late fall. In contrast with the results with mature trees, however, the late-spring and midsummer applications resulted in a higher nitrogen level in the foliage, which in turn was associated with greater growth. Because of the variable production behavior associated with young trees for the first few years of bearing, little significance could be attached to the production performance of these trees during the course of these studies. It is reasonable to expect, however, that differences in growth rate would ultimately be reflected in fruit production.

The color records on Rome Beauty indicated a very close association between nitrogen content of the foliage, particularly in late summer, and fruit color. Crops of the Starking Delicious and York Imperial in this young orchard were too light and variable to make color records significant.

Sod, which might compete with the tree for applied nitrates, was removed prior to the start of the experiments. Results on the nitrate movement in the soil suggest possible advantage in applying nitrogen in the fall for trees growing in sod. When the nitrate was applied

in the fall most of it was in the second and third feet of soil the following spring, which would be below the dense mass of grass roots in the first foot of soil. Most of the nitrate applied in the spring remained in the top foot throughout the summer or until absorbed by the tree. These results indicate that trees growing in sod probably will obtain more of the nitrogen if it is applied in the fall. Experimental data bearing specifically on this point were not obtained.

The results on nitrate disappearance also emphasize that even the most available forms of nitrogen are taken up rather slowly by trees and that some absorption probably occurs throughout the year. Approximately half of the total nitrate applied in early spring was still recoverable in soil samples taken in late July, and approximately one-fourth could be recovered at the end of the growing season. The same general situation held with other times of application. Thus, enough nitrogen may be absorbed within a relatively short time to affect foliage color, but a relatively long period is required before most of the applied nitrogen is taken up by the tree. Maximum rate of absorption of nitrates by the trees was in early spring during the period of expansion of the leaf system, although rapid uptake occurred in midsummer if the trees were not previously well supplied with nitrogen.

CONCLUSIONS AND SUMMARY

Investigations were conducted through 4 years to determine the relation of season of nitrogen application to response in mature apple trees. Under the conditions of these experiments the following conclusions are indicated:

(1) On relatively old York Imperial and Delicious apple trees fruit set, color, and yield were not influenced by season of nitrogen application.

(2) Greater downward leaching of nitrate occurred when sodium nitrate was applied to the soil in early or late fall than when applied in spring or midsummer. With mature trees this greater leaching into the second and the third foot of soil apparently was not reflected in less nitrogen intake and tree growth.

(3) Nitrogen content of blossoms, shoots, leaves, bark, wood, and roots, which was determined throughout these experiments, did not indicate any large or consistent differences due to time of nitrogen application.

(4) After 4 years of treatment of mature apple trees no difference was found between fall and spring applications in the nitrogen reserve contained in the various tissues of the tree.

(5) Results with mature apple trees strongly suggest that, if the level of nitrogen metabolism is satisfactorily maintained within the tree, it makes little difference at what season of the year the soil supply is replenished.

Tests were conducted during 5 years with young Starking Delicious, Rome Beauty, and York Imperial trees. Late-spring and mid-summer applications of nitrate nitrogen resulted in a higher nitrogen level in the foliage, which in turn was associated with greater growth and in Rome Beauty with less fruit color. No significant differences were found in nitrogen content of foliage, in growth, or in color of fruit when nitrogen was applied in early spring, early fall, or late fall.

LITERATURE CITED

(1) ALDRICH, W. W.
    1932. NITROGEN INTAKE AND TRANSLOCATION IN APPLE TREES FOLLOWING FALL, WINTER AND SPRING SODIUM NITRATE APPLICATIONS. Amer. Soc. Hort. Sci. Proc. (1931) 28: 532–538.
(2) BALLARD, W. S., and VOLCK, W. H.
    1914. WINTER SPRAYING WITH SOLUTIONS OF NITRATE OF SODA. Jour. Agr. Res. 1: 437–444, illus.
(3) BATJER, L. P., and DEGMAN, E. S.
    1940. EFFECTS OF VARIOUS AMOUNTS OF NITROGEN, POTASSIUM, AND PHOSPHORUS ON GROWTH AND ASSIMILATION IN YOUNG APPLE TREES. Jour. Agr. Res. 60: 101–116, illus.
(4) ―――― MAGNESS, J. R., and REGEIMBAL, L. O.
    1943. NITROGEN INTAKE OF DORMANT APPLE TREES AT LOW TEMPERATURE. Amer. Soc. Hort. Sci. Proc. 42: 69–73.
(5) BOYNTON, D., and BURRELL, A. B.
    1944. EFFECTS OF NITROGEN FERTILIZER ON LEAF NITROGEN, FRUIT COLOR AND YIELD IN TWO NEW YORK MCINTOSH APPLE ORCHARDS, 1942 AND 1943. Amer. Soc. Hort. Sci. Proc. 44: 25–30.
(6) CHILDERS, N. F.
    1938. THE INFLUENCE OF CERTAIN NUTRIENTS ON THE PHOTOSYNTHETIC ACTIVITY OF APPLE LEAVES. (Abstract) Amer. Soc. Hort. Sci. Proc. (1937) 35: 253–254.
(7) CULINAN, F. P., SCOTT, D. H., and WAUGH, J. G.
    1939. THE EFFECTS OF VARYING AMOUNTS OF NITROGEN, POTASSIUM, AND PHOSPHORUS ON THE GROWTH OF YOUNG PEACH TREES. Amer. Soc. Hort. Sci. Proc. (1938) 36: 61–68, illus.
(8) FREAR, L. E. H., and ANTHONY, R. D.
    1943. THE INFLUENCE OF DATE OF SAMPLING ON THE VALUE OF LEAF WEIGHTS AND CHEMICAL ANALYSES IN NUTRITION EXPERIMENTS WITH APPLE TREES. Amer. Soc. Hort. Sci. Proc. 42: 115–122, illus.
(9) HEINICKE, A. J.
    1935. PHOTOSYNTHESIS IN APPLE LEAVES DURING LATE FALL AND ITS SIGNIFICANCE IN ANNUAL BEARING. Amer. Soc. Hort. Sci. Proc. (1934) 32: 77–80.
(101) HOAGLAND, R. R., and ARNON, D. I.
    1941. PHYSIOLOGICAL ASPECTS OF AVAILABILITY OF NUTRIENTS FOR PLANT GROWTH. Soil Sci. 51: 431–444.
(11) HOOKER, H. D., Jr.
    1922. THE SEASON OF APPLICATION OF NITROGENOUS FERTILIZER AS AFFECTING THE CHEMICAL COMPOSITION OF SPURS AND BARK. Amer. Soc. Hort. Sci. Proc. (1921) 18: 150–152.
(12) MAGNESS, J. R., BATJER, L. P., and REGEIMBAL, L. O.
    1940. CORRELATION OF FRUIT COLOR IN APPLES TO NITROGEN CONTENT OF LEAVES. Amer. Soc. Hort. Sci. Proc. (1939) 37: 39–42, illus.
(13) ―――― and REGEIMBAL, L. O.
    1939. THE NITROGEN REQUIREMENT OF THE APPLE. Amer. Soc. Hort. Sci. Proc. (1938) 36: 51–55.
(14) OVERHOLSER, E. L., and OVERLEY, F. L.
    1940. THE EFFECT OF TIME OF NITROGEN APPLICATION UPON THE RESPONSE OF JONATHAN APPLES. Amer. Soc. Hort. Sci. Proc. (1939) 37: 81–84.
(15) SCHRADER, A. L., and AUCHTER, E. C.
    1928. THE COMPARATIVE EFFECTS OF DIFFERENT NITROGEN FERTILIZERS ON BEARING APPLE TREES LOW IN VIGOR. Amer. Soc. Hort. Sci. Proc. (1927) 24: 229–233.
(16) SMITH, G. E.
    1937. NITROGEN CONTENT AND GROWTH RESPONSE FROM FALL AND SPRING FERTILIZER APPLICATIONS TO APPLE TREES. Amer. Soc. Hort. Sci. Proc. (1936) 34: 133–136.
(17) WEINBERGER, J. H., and CULLINAN, F. P.
    1935. NITROGEN INTAKE AND GROWTH RESPONSE IN PEACH TREES FOLLOWING FALL AND SPRING FERTILIZER APPLICATIONS. Amer. Soc. Hort. Sci. Proc. (1934) 32: 65–69.

INHERITANCE OF RESISTANCE TO BACTERIAL WILT IN TOBACCO [1]

By T. E. Smith, formerly *associate pathologist*, and E. E. Clayton, *senior pathologist, Division of Tobacco, Medicinal, and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

The occurrence of genetic resistance to bacterial (Granville) wilt, caused by *Bacterium solanacearum* E. F. Sm., in tobacco (*Nicotiana tabacum* L.) has been reported.[2] Different levels of resistance have been found in certain genotypes: Low resistance in numerous strains including certain flue-cured varieties, moderate resistance in T. I.[3] 79A[4] and Turkish Xanthi, and high resistance in 79–X and T. I. 448A.[5] The last-named genotype had no objectionable growth ·or quality characteristics. In later work Oxford 26,[6] a flue-cured variety with high wilt resistance and quality, was developed by selection from a cross of T. I. 448A × 400. Adequate data have been accumulated from the breeding work to permit a detailed report on the inheritance of wilt resistance. Such information should be of aid in solving the problem of combining resistance to wilt with resistance to other diseases and in coping with other problems arising from the development and use of wilt-resistant varieties.

MATERIAL AND METHODS

F_1 generations were grown for seed increase in the greenhouse during the winter. Seedlings of the F_2 and later generations were grown in steam-sterilized beds and transplanted into naturally contaminated soil in the field. Fertilization and cultural methods were similar to those used for flue-cured tobacco. Sites for test plantings were selected on the basis of uniformity trials in which 95 percent or more of known-susceptible varieties were killed by wilt. Single-row plots (fig. 1) of 25 to 150 plants were grown in completely randomized blocks, grouped according to generation or parentage so that closely related strains occurred in the same section of the field. The number

---

FIGURE 1.—*A*, Strain DSPA, selected from hybrids of Davis Special × Pinkney Arthur; *B*, wilt-susceptible variety; *C*, F₅ of (T. I. 448A × 400) × 101.

of replications ranged from 3 to 5 in most experiments. The amount of wilt was determined 12 or more weeks after transplanting, and was recorded as a disease index in which 0 equaled no symptoms and 100 the killing of all plants. The disease index was a weighted average of a uniform number of plants from each replication graded into 4 groups with assigned values of 0, 25, 50, and 100.

UTILIZATION OF THE LOW WILT RESISTANCE FOUND IN CERTAIN FLUE-CURED VARIETIES

Low resistance to wilt was reported by Garner, Wolf, and Moss.[7] Low resistance has also been found in numerous introduced genotypes [8] and in the flue-cured varieties Davis Special, Pinkney Arthur, and 400. Efforts to increase this resistance by line selection failed, but some success resulted from selection within intervarietal hybrids. Under conditions such that highly susceptible varieties usually had a disease index of 95 or more, Davis Special and Pinkney Arthur had indices that ranged from 74.7 to 82.0 (table 1). The F₁ of Davis Special × Pinkney Arthur was completely susceptible, but resistant plants occurred in the F₂. These were selfed, and there were established a number of F₃ lines that had significantly less wilt than either parent (table 1). After selection through the F₅, a composite F₆ (fig. 1) had a disease index of 55.8, as compared with 98.6 for a susceptible variety. This material was designated DSPA. In trial plantings on farmers' fields it had enough resistance to mature a full

[7] GARNER, W. W., WOLF, F. A., and Moss, E. G.   THE CONTROL OF TOBACCO WILT IN THE FLUE-CURED DISTRICT.   U. S. Dept. Agr. Bul. 562, 20 pp., illus. 1917.
[8] See footnote 2, p. 27.

crop under conditions of moderate wilt severity. However, on the heavily infected experimental plots the DSPA strain showed much less resistance than T. I. 448A, which generally had a disease index of approximately 10.

TABLE 1.—*Wilt indices of Davis Special and Pinkney Arthur tobaccos and of the hybrids and selections, 1941–43*

[0=No symptoms of wilt and 100=killing of all plants]

Genotype	Disease index of indicated generation			
	F_1	F_2	F_3	F_4
Davis Special parent			[1] 80 7	
Pinkney Arthur parent			[1] 74. 7	[1] 82 0
Davis Special × Pinkney Arthur	96 7	90 0		
Pinkney Arthur × Davis Special		94 0		
Selection 1			22 6	34 5
Selection 2			29 0	39 5
Selection 3			32 0	40 5
Selection 4			32 6	67 0
Selection 5			34 3	73 0
Selection 6			53 3	79 5
Selection 7			57. 8	79.5
Selection 8			58 3	
Selection 9			60 7	
Selection 10			66 3	
Selection 11			67.0	
Least significant difference ($P=0$ 01)			30 5	25 7

[1] From plantings of the regular variety indicated.

ATTEMPTS TO DEVELOP AN IMMUNE LINE

The additive nature of wilt resistance occurring in strains with moderate resistance was shown by selection of 79–X from the cross T. I. 79A × Turkish Xanthi.[9] An effort was made to find whether the high resistance in T. I. 448A and 79–X could be accumulated to produce immunity. In a cross of moderately resistant strain × highly resistant strain (DSPA and a hybrid having the full resistance of T. I. 448A, table 1), it was not possible to recover more resistance than that of T. I. 448A by selection in the F_2 and F_3. The cross between 2 highly resistant strains was of the most interest. The strains 79–X and T. I. 448A had disease indices of approximately 10, and a distinct reduction of this value would have approached immunity. The F_1 population from T. I. 448A × 79–X had significantly more wilt than either parent, indicating that the 2 strains had different genes for resistance. A total of 117 F_2 plants that appeared free from wilt were tested as F_3 lines in 1941. Of these, 13 had a lower disease index than T. I. 448A. Healthy plants were selected from the most resistant lines, and this procedure was continued through the F_6. The final results were negative, as strains with more resistance than T. I. 448A were not recovered. The reason for the failure may have been that critical wilt severity did not occur regularly enough for effective selection within the disease-index range 10 to 0.

[9] See footnote 2, p. 27.

INHERITANCE OF RESISTANCE IN CROSSES OF T. I. 448A WITH FLUE-CURED VARIETIES

T. I. 448A was crossed with several flue-cured varieties. Data were obtained on the inheritance of resistance in 8 generations of the original cross (table 2). The F_1 was almost as susceptible as the susceptible parents, with disease indices of 90.4 and 94.7, respectively. Thus resistance was recessive. The F_2 had less wilt than the F_1 or the susceptible parents. Individual F_2 plants were selected for resistance; of 166 such selections tested as F_3 lines, 7 were highly resistant. The other 159 gave a distribution all the way to complete susceptibility. Selection in the F_3 and F_4 generations was required to eliminate all susceptible lines. Continued selection in the F_5 through the F_7 maintained resistance equal to that of T. I. 448A but did not increase it.

TABLE 2.—*Wilt indices of hybrids of resistant T. I. 448A × susceptible varieties of tobacco, 1941–43*

[0=No symptoms of wilt and 100=killing of all plants]

Genotype	Total tests	Tests with disease index [1] of—										Average disease index
		1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	
		Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
Resistant parent (T. I. 448A)	12	3	9	0	0	0	0	0	0	0	0	12 5
Susceptible parents	23	0	0	0	0	0	0	0	0	2	21	94 7
F_1	5	0	0	0	1	0	0	0	0	3	2	90 4
F_2	7	0	0	0	0	0	2	1	1	3	0	71 8
F_3	166	7	22	23	26	28	18	23	11	7	1	42 8
F_4	42	11	9	7	7	4	4	0	0	0	0	23 8
F_5	11	3	6	2	0	0	0	0	0	0	0	13 7
F_6	23	23	0	0	0	0	0	0	0	0	0	3 1
F_7	4	3	1	0	0	0	0	0	0	0	0	10 5
F_8	8	3	4	1	0	0	0	0	0	0	0	15 6
Resistant F_2 × susceptible parent:												
F_2	13	0	0	0	0	0	5	2	2	2	2	70. 6
F_3	169	1	8	15	15	28	31	29	21	15	6	56 8
F_4	67	28	18	17	2	1	1	0	0	0	0	15. 2
F_5	38	11	14	12	1	0	0	0	0	0	0	17. 0
F_6	17	2	13	2	0	0	0	0	0	0	0	15. 8

[1] Each test was a replicated planting of 75 to 300 individuals. Over 52,000 plants are represented in the entire table.

Resistant F_3 selections were backcrossed to susceptible varieties, and data on the F_2 through the F_6 are also summarized in table 2. The pattern was similar to that for the results obtained from the original cross. Successive generations of the original cross and the first backcross had disease indices, respectively, as follows: F_2, 71.8 and 70.6; F_3, 42.8 and 56.8; F_4, 23.8 and 15.2; F_5, 13.7 and 17.0; F_6, 3.1 and 15.8. The results showed that the full resistance of T. I. 448A was recovered from the original and the first backcross even though resistance was controlled by a number of genes. Inheritance of resistance in hybrids of 79-X × flue-cured varieties followed the same pattern in less extensive data.

RELATION OF SUSCEPTIBLE PARENT TO THE RECOVERY OF
RESISTANCE

Attention was also given to the effect of different flue-cured varieties
on the recovery of resistance in the hybrids. Data on the F_1, F_2, and
F_3 of hybrid families arising from crosses of T. I. 448A with 6 flue-
cured varieties are shown in table 3. All F_1 populations were highly
susceptible. The F_2 populations from crosses with Davis Special and
400 had significantly less wilt. In the F_3, the Davis Special family
had few resistant selections but the 400 family had many resistant
selections. Of 55 F_3 lines of 400 parentage, 19 had a disease index of
20 or less and hence were highly resistant. In table 3, 141 F_3 lines are
reported; very high resistance (disease index of 10 or less) occurred in
7 of these; of these 7, 5 came from the cross with 400. The superior
combining ability of this variety, which resulted in numerous segre-
gates with wilt resistance, was a major factor in the development of a
commercial flue-cured tobacco with high resistance to wilt.

TABLE 3.—*Effect of crossing T. I 448A with susceptible varieties of tobacco on recovery of resistance to wilt, 1941–43*

[0=No symptoms of wilt and 100=killing of all plants]

Susceptible variety	Average disease index of—		F_3 lines [1] recovered with indicated disease index									
	F_1	F_2	1–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
			Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
Gold Dollar	98 9	82 0	1	5	5	5	8	3	3	2	1	1
400	89.7	60 0	5	14	14	9	4	3	5	1	0	0
Davis Special		58 5	0	1	0	4	6	8	9	2	4	0
Cash	85 3	82.0	1	0	1	1	3	2	0	1	0	0
Bonanza	87 3	79.5	0	0	0	3	1	1	1	0	0	0
Virginia Bright Leaf	91.0	81.5	0	0	0	1	1	0	0	0	1	0
Least significant difference ($P=0.01$)	([2])	11 2										

[1] Each line was a planting replicated 3 times from a selfed F_2 selection.
[2] Not significant.

DISCUSSION

Wilt resistance from three separate sources has been investigated
with respect to degree and inheritance. Certain flue-cured varieties,
Davis Special, Pinkney Arthur, and 400, had low resistance. A
cross between two of these produced, after selection through the F_5,
a genotype with a disease index of about 55 as compared with 75 to
about 80 for the parents and 95 to 100 for susceptible varieties. Some
years ago obtaining this degree of resistance would have been regarded
as a major achievement. At present the higher resistance obtained
in foreign collections is preferred. The best resistant genotypes ob-
tained were 79–X, which was developed from the cross of moderately
resistant T. I. 79A with Turkish Xanthi, and T. I. 448A. Both gave
disease-index values of about 10. When 79–X was used as the source
of resistance, the resistant segregates tended to have small leaves of
poor quality. T. I. 448A, on the other hand, produced highly resist-
ant lines with good agronomic characters. The resistance in each
case was recessive and controlled by multiple genes. Resistance in

T. I. 448A and that in 79–X were not identical, because the F_1 of a cross of these two was more susceptible than either parent. An effort was made to develop an immune genotype by selection in the hybrids of T. I. 448A × 79–X. The attempt failed, presumably because it was not possible to select effectively within a disease-index range of 10 to 0. Therefore, resistant genotypes exist as (1) T. I. 448A, (2) 79–X, selected from a cross of T. I. 79A × Turkish Xanthi, and (3) DSPA, selected from a cross of Davis Special × Pinkney Arthur. T. I. 448A was the resistant breeding stock in the development of Oxford 26. The other genotypes constitute a reserve of possible value should physiologic specialization occur in the pathogen.

Oxford 26 was released for increase of seed stocks 5 years after T. I. 448A was crossed with flue-cured varieties. Factors that contributed to its rapid development were (1) lack of objectionable quality or growth characters in T. I. 448A, (2) recessive resistance, (3) occurrence of increased numbers of resistant segregates in crosses with 400, and (4) growth of large populations under such severe disease conditions that the few highly resistant segregates could be picked out in each generation. Over 52,000 plants were grown in the F_2 through the F_6 generations of the original and first backcrosses of T. I. 448A × flue-cured varieties; 532 individual-plant selections were tested. Only 1 selection was of sufficient value to be released as a variety, but 5 genotypes had potential value. It is significant that all 5 of the potentially valuable selections were of 400 parentage. Hence, rapid progress was possible because of the combining performance of 400, which assisted in the recovery of resistance, and because of the growth of large populations to facilitate recovery of quality and growth characters. Resistance to other tobacco diseases is controlled by multiple genes. The results of these studies on wilt resistance may suggest methods of obtaining rapid progress with other genetic material of this type.

SUMMARY

Resistance to bacterial wilt was recessive and controlled by multiple genes. The flue-cured varieties Davis Special and Pinkney Arthur were slightly resistant. It was possible to select a moderately resistant genotype from a cross of the two. Genotypes possessing high resistance were T. I. 448A and 79–X. Segregates from crosses of T. I. 448A × flue-cured varieties were much superior in growth and quality characters to segregates from similar crosses in which 79–X was the source of resistance. Of the flue-cured varieties used as parents, variety 400 produced the most progeny with high resistance and good agronomic characters. After crossing T. I. 448A with a susceptible tobacco it was necessary to continue selection to the F_5 generation before the full resistance was recovered. This resistance was not increased beyond the T. I. 448A level by selection through the F_7 generation. After backcrossing resistant segregates to susceptible tobacco the full T. I. 448A resistance was again recovered in the F_5 generation. Because of high resistance, yield, and quality standards, rapid progress was possible only through the use of large populations and conditions permitting critical evaluation. Only 5 potentially valuable genotypes have been retained from 52,000 hybrid plants of T. I. 448A parentage.

THE PRESCOTT SCALE (MATSUCOCCUS VEXILLORUM) AND ASSOCIATED ORGANISMS THAT CAUSE FLAGGING INJURY TO PONDEROSA PINE IN THE SOUTHWEST[1]

By HOWARD L. McKENZIE, formerly *assistant entomologist, Division of Forest Insect Investigations, Bureau of Entomology and Plant Quarantine*, and L. S. GILL, *senior pathologist*, and DON E. ELLIS, formerly *assistant pathologist, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

In 1918 a so-called twig blight of young ponderosa pine (*Pinus ponderosa* Laws.) was noted in the Prescott National Forest, in Arizona. At that time the twig killing, or flagging, was found only on an area of about 500 acres near the city of Prescott. Investigations were begun in 1919 by W. H. Long, of the Bureau of Plant Industry, who thought the trouble might be caused by a species of *Cenangium*, presumably an indigenous and relatively harmless fungus. Long's unpublished records indicate that until 1933 the flagging showed considerable annual variation in intensity, but that the size of the affected area did not appear to be increasing beyond the original 500 acres.

Suddenly, in 1933 the twig killing reached epidemic proportions and was abundant over an area of about 30,000 acres in the Prescott National Forest, and two new heavily flagged areas were found in southwestern New Mexico. The Bureau of Plant Industry immediately started intensive work on the problem, primarily to determine whether some introduced organism might be involved, and in 1935 the Bureau of Entomology and Plant Quarantine began an investigation of the associated insects.[3] The research was continued until the spring of 1938, when it became clearly evident that a scale insect,

Matsucoccus vexillorum Morrison,[4] was the primary cause of the trouble, that there was no immediate danger to the pine forests as a whole, and that local damage was of little economic importance.

OCCURRENCE OF MATSUCOCCUS AND FLAGGING INJURY

In 1934 a survey of the ponderosa pine forests of the United States was conducted by the Division of Forestry Pathology of the Bureau of Plant Industry, in cooperation with the Civilian Conservation Corps and the Forest Service. No new flagged areas were discovered in the course of that work but more intensive surveys, made subsequently, revealed that flagging existed at a number of widely scattered localities in Arizona and New Mexico. The known distribution of the *Matsucoccus* scale and flagging injury in 1942 is shown in figure 1. In 1943 several hundred acres of heavily flagged trees were discovered 10 to 15 miles west of Durango, Colo.

Flagging at Prescott increased rapidly from 1932 to 1934, then declined markedly from 1935 to 1937, but rose again slightly during 1938 and 1939. Flagging in 1938 was estimated to be about half as extensive as in 1934, and in 1939 it was about 37 percent of that in 1934. In 1940 it was extremely light. In 1946 it was moderate throughout the forest. Because of the removal and destruction of much affected material in the course of control work on the forest, these figures cannot be considered as representative of undisturbed natural conditions.

EFFECTS OF TWIG FLAGGING

Flagging usually occurs on trees less than 75 years old and is most conspicuous in dense stands of saplings or seedlings. Trees are rarely killed, although in years of heavy attack so many twigs are killed that at first glance many trees appear to be dead. Estimates of mortality among affected trees at Prescott during 1933 and 1934, years of severe flagging, ranged from 1 to 2 percent. A tally of 8,593 trees on 33 sample strips in the Prescott National Forest showed an average annual mortality of 0.13 percent for both 1938 and 1939. Most of the mortality was among trees less than 6 feet high, and no trees over 7 inches in diameter at 4½ feet above ground were killed.

A rather heavily flagged tree is shown in figure 2. Usually all the foliage on a flagged twig is killed, although sometimes green needles will remain behind the dead tip. The foliage of characteristically flagged twigs first turns light green, and the needles continue to fade, usually from their base outward, turning straw-yellow and finally light brown. Ordinarily the needles persist only for several months after the death of the twig, but sometimes they remain attached to it for more than a year.

Most of the injury appears in the spring, although in most infected areas a few flags may be seen early in the winter. The heavy period of twig killing at Prescott seems to coincide with warm, dry weather, which normally occurs between the middle of April and the middle of

[4] McKenzie, H. L. THE SEASONAL HISTORY OF *Matsucoccus vexillorum* MORRISON (HOMOPTERA: COCCOIDEA: MARGARODIDAE). Microentomology 8: 42–52, illus. 1943.
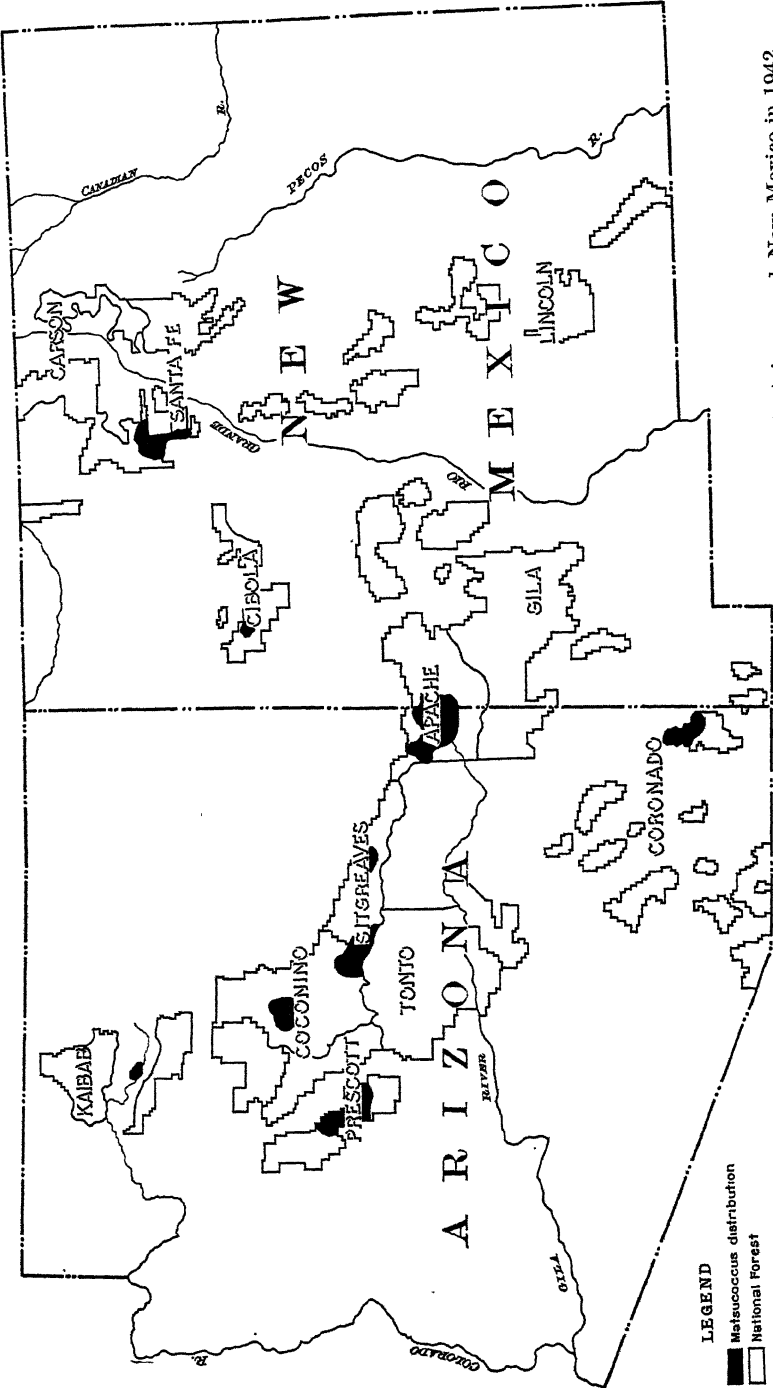
FIGURE 1.—Distribution of *Matsucoccus vexillorum* and flagging injury to ponderosa pine in Arizona and New Mexico in 1942. Affected areas are indicated in black.

May. Dry, warm winters are conducive to early flagging, which in some years has been conspicuous by the middle of January. As a rule, there is a marked drop in twig dying after the spring peak is
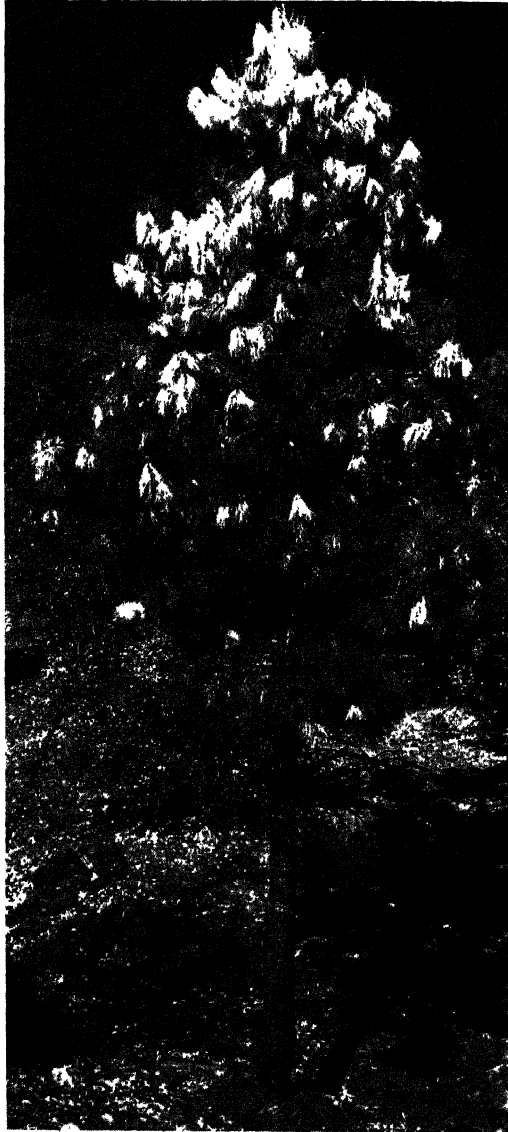
FIGURE 2.—Flagged ponderosa pine, Prescott National Forest. The dead twigs, or flags, are conspicuous by their light-colored, drooping foliage.

reached, and new killing after the middle of June is rare. Typical flagged twigs die, almost without exception, before the current year's needles emerge from the buds.

The old dead twigs from which the needles have fallen give a peculiar "staghorn" appearance to a tree, and trees that have been attacked for several successive years exhibit a singular crooked-branch habit and a thin crown (fig. 3). Flagging of the terminal shoot is not uncommon, and its frequent occurrence causes the tree to become badly malformed.

There seems to be no important relation between flagging and tree vigor or forest site. Vigorous, as well as suppressed, trees and twigs

FIGURE 3.—Ponderosa pine that has a "staghorn" appearance because it has been successively flagged for a number of years.

have been attacked on a wide variety of forest sites. It is possible that periods of severe twig killing are correlated to some extent with climatic conditions, but the relationship is not fully understood. The epidemic years of 1933 and 1934 at Prescott were below normal in precipitation and marked the last 2 years of a 4-year period of steadily declining tree growth.

HISTOLOGY OF AFFECTED TWIGS

Affected twigs invariably exhibit lesions, which are readily detected by the presence of necrotic bark. Lesions are most common on the

3- and 4-year-old growth, but may develop on any foliated part of the stem except the 1-year-old growth. The bark tissue turns brown and later becomes impregnated with resin. Extensive field observations indicate that, when bark necrosis is manifest during the fall in the form of small brownish areas in the living phloem, it may lead to flagging in the following winter or spring. The approximate center of these necrotic spots is usually a fascicle trace. The senior writer [5] has found that *Matsucoccus vexillorum* tends to settle at the base of the needle fascicles.

The necrotic spots enlarge and deepen in the phloem during the winter. When they reach the cambium and completely girdle it, the twig is killed. Fortunately pine trees seem to be well equipped to combat the progress of necrosis by walling off the diseased tissue with a thin layer of cork. Owing to this warding-off process, a great many lesions become arrested for every one that is able to cause a flag under natural conditions.

Late in the summer of 1937 tags were fastened to 77 twigs, each of which showed 1 or more bundles of dead or paling needles, believed to indicate a necrotic spot in the bark. By April of the following year none of the twigs had flagged. An examination of some of the twigs at that time showed that, of 169 fascicles containing paling needles marked on 34 twigs, 164 were associated with lesions only 1 of which did not appear to have been arrested. Of the twigs left undisturbed, none died later, a fact which suggests that all the lesions on them were arrested. The single active necrotic spot might have developed into a girdling lesion. The trees used in this experiment were in a heavily flagged locality, and several unmarked twigs on them flagged both before and after the experiment.

Where necrosis is arrested early in the development of a lesion, and does not reach the cambium, the effect is analogous to the formation of a bark plate. Normal phloem tissue continues to develop below the cork layer, and in time the affected tissue is sloughed off, leaving no trace of its former existence. This condition is illustrated in figure 4. Where necrosis reaches the cambium before being walled off by a cork layer, one of two things may happen—either cambial activity may be temporarily disrupted beneath the necrotic area, which is subsequently walled off, or an arc of the cambium may be killed, leaving a permanent record of the injury. Figure 5 illustrates these conditions.

All observations indicate that the progress of bark necrosis is limited to the season of its origin; that is, a lesion that originates in the fall either kills the twig by the following spring or is permanently arrested.

In addition to the bark necrosis, lesions are generally associated with necrotic fascicle traces. The latter turn brown, and the discoloration extends well into the wood, commonly as far as the central pith. When a small lesion circumscribes a single needle trace, this needle trace is almost invariably necrotic. In extensive lesions, notably lethal ones involving several years' growth, some of the fascicle traces are necrotic, whereas others show no indication of a pathological condition.

---

[5] See footnote 4, p. 34.

The xylem beneath necrotic bark generally becomes infiltrated with resin after necrosis is well advanced. Where partial lesions exist, the resined wood is wedge-shaped, a fact suggesting that it is

FIGURE 4.—Ponderosa pine twig with outer bark removed, showing extent of several small arrested lesions in living phloem.

bordered radially by medullary rays. In a girdling lesion a length of the entire wood cylinder may be impregnated with pitch. A lesion involving several inches of stem is usually accompanied by completely pitch-soaked wood for only a relatively short distance from its basal extremity.
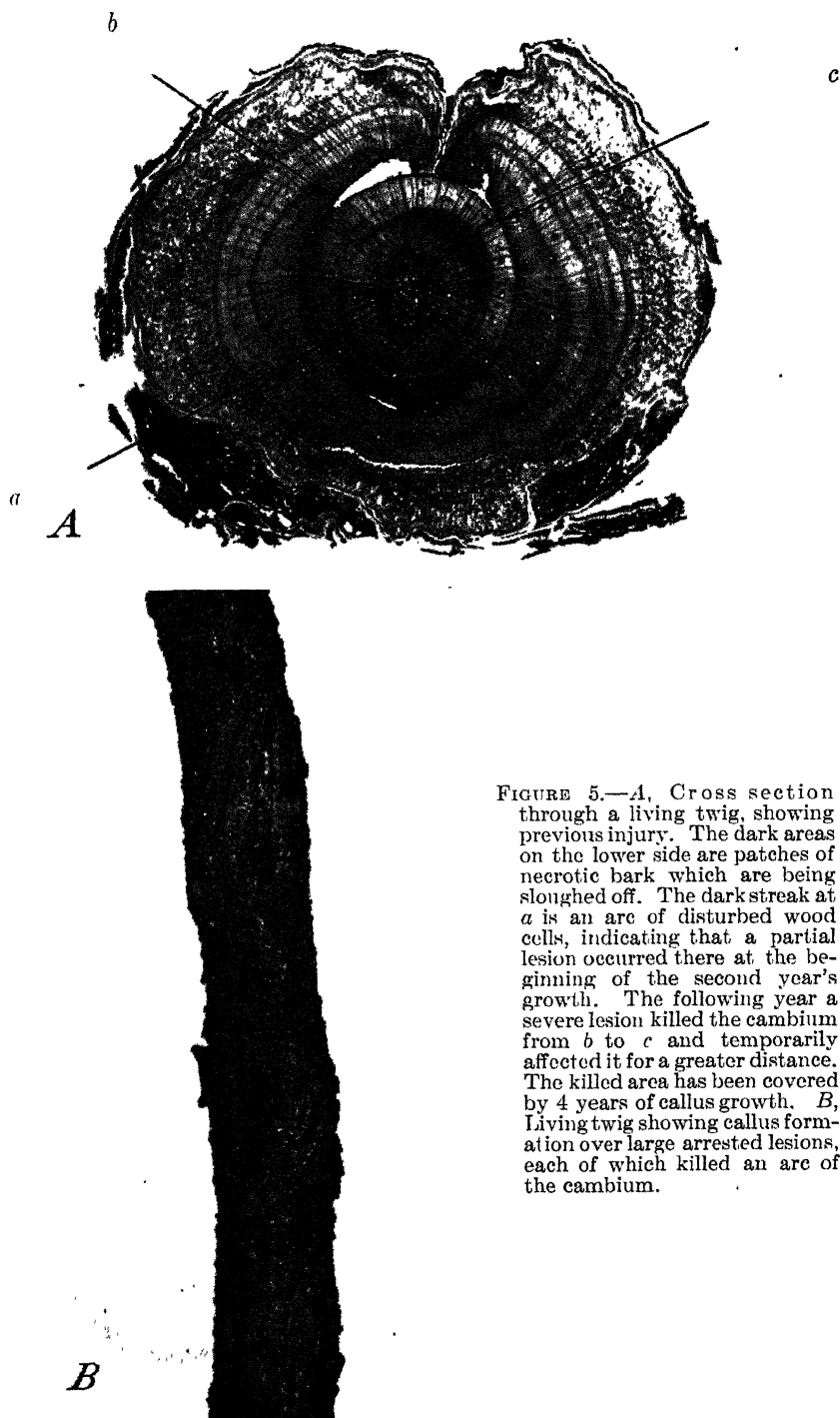
FIGURE 5.—*A*, Cross section through a living twig, showing previous injury. The dark areas on the lower side are patches of necrotic bark which are being sloughed off. The dark streak at *a* is an arc of disturbed wood cells, indicating that a partial lesion occurred there at the beginning of the second year's growth. The following year a severe lesion killed the cambium from *b* to *c* and temporarily affected it for a greater distance. The killed area has been covered by 4 years of callus growth. *B*, Living twig showing callus formation over large arrested lesions, each of which killed an arc of the cambium.

The physiological processes leading to flagging have not been studied, but general observations indicate that it is probably the result of rapid necrosis. Many twigs in the process of flagging show necrotic bark for several inches, and often over several years' growth, while the needles are still green and succulent. Ponderosa pine twigs have been observed to remain alive for many months—some even for 2 years—after the removal of a band of bark about an inch wide. This suggests that something more than cambial girdling alone is necessary to cause flagging.[6]

EXPERIMENTS WITH MATSUCOCCUS
ARTIFICIAL INFESTATION OF TWIGS

The first tests to establish the nature of the relation of *Matsucoccus vexillorum* to flagging were begun in April 1936 (see footnote 3, p. 33). At that time 10 ponderosa pines were artificially infested with scales collected from heavily flagged trees in the forest. Egg masses of the scale, still attached to the branch nodes, were placed in small, thimble-shaped, copper-screen baskets, which were then fastened securely onto the twigs with fine copper wire (fig. 6). A total of 220 twigs were infested, and during the following season 46 percent of them flagged. In 1938, 5 additional twigs on these trees flagged, and the next year 4 more died.

In the spring of 1937 an experimental plot was established on the Wolf Creek area of Prescott National Forest. This plot (No. 1) included 15 trees, presumably free from natural scale attack and branch flagging, which were artificially infested at this time. On some of the trees the scale egg masses were placed on the branches by means of a camel's-hair brush, and on the remainder copper-screen baskets were employed. Seventy branches on 7 of these experimental trees were used as checks, empty copper baskets being attached in the manner used for introducing the scale insects.

Early in the spring of 1938, before there was much evidence of flagging, each infested twig was carefully examined in the field by the use of a 10× power hand lens to determine the population density of preadult scales. Population-density classifications were set up as heavy, medium, and light. The results of the work are included in table 1. The significant feature of these population studies is that, although 80 percent of the heavily infested branches flagged, those carrying only a light infestation or no scales, as well as the 70 check

---

[6] After examining this twig-blight area in April 1936, F. C. Craighead pointed out the similarity of this injury to "red twig" of balsam and other conifers, caused by mechanical injuries resulting in the destruction of the phloem. He said:

It [the blighting of the twigs] is a most common phenomenon following logging operations where the pine sawyers (*Monochamus* spp.) breeding in the slash become abundant. These beetles gnaw off small areas of bark on living twigs. It is also common for several years after severe hail storms have caused bruised areas on the bark. The simplest physiological explanation seems to be based on the fact that most conifers secrete resin after a wounding of the phloem and cambium. This infiltration of resin often involves many annual layers of wood (turpentine faces) and stops all conduction through the area involved. In the case of the twigs a small sector to almost an entire cross section may become infiltrated with resin and conduction is greatly inhibited. From 1 to 5 years later, during periods of unusual stress, particularly following dry winter winds, when conduction is insufficient, the leaves turn brown.

Craighead believes that this type of injury can explain twig blight in the Prescott National Forest following the initial killing of the phloem by the scale. Other species of sucking insects apparently kill the twigs of conifers in much the same manner.

twigs, remained alive. Fourteen additional twigs on the trees in this plot flagged in 1939. It is probable that where no insects became established most of the eggs had hatched and the young larvae had already moved elsewhere before the egg masses were collected and placed on the twigs.

FIGURE 6.—Copper-screen baskets used to carry out the artificial infestation of ponderosa pine with *Matsucoccus vexillorum* scales.

TABLE 1.—*Results of the artificial infestation with Matsucoccus scale of 15 ponderosa pines in plot 1, Prescott National Forest, in 1937*

Population density, spring 1938	Twigs examined	Twigs flagged, summer 1938
	Number	*Percent*
None	88	0
Light	18	0
Medium	14	36
Heavy	30	80
Total	150	19

In the spring of 1938 two additional plots were laid out in the Prescott National Forest—plot 2 in the Wolf Creek area and plot 3 in the Copper Basin Divide. The Wolf Creek plot, at the time of establishment, was in a supposedly flag-free area, although Forest Service records indicated that some affected material had been removed in previous years. According to the Forest Service records,

the Copper Basin Divide plot had never shown any flagging. Most of the trees on this plot were vigorous "orchard" types. On plot 2, 90 trees and on plot 3, 60 trees were artificially infested with *Matsucoccus* scales. Table 2, which is based on data from all except 21 trees in plot 2, which were reserved for pathological studies by the Division of Forest Pathology, shows that from 36 to 54 percent of the artificially infested branches had flagged by the summer of 1939. Figure 7 shows the extent of flagging on one of the trees in plot 3.

FIGURE 7.—Tree No. 10, in plot 3, Copper Basin Divide, artificially infested with the *Matsucoccus vexillorum* scale in the spring of 1938. Eighty-two percent of the infested branches on this tree flagged in 1939.

TABLE 2.—*Results of the Artificial Infestation of Ponderosa Pine With the Matsucoccus Scale in Plots 2 and 3, Prescott National Forest in 1938*

Plot No.	Trees involved	Average height	Average diameter	Branches infested	Branches flagged, 1939
	Number	*Feet*	*Inches*	*Number*	*Percent*
2	69	12	2.8	702	36
3	60	16	3.8	628	54

In plot 4, established in 1938 in the Lynx Creek area, 80 naturally infested and flagged trees were marked, and 231 twigs carrying various numbers of adult females and egg masses were tagged for future observation. While the tendency for the twigs to flag was in direct proportion to the density of adult females and egg masses, the trend was less marked than in plots 2 and 3, where the preadult, or feeding stage, was used as an index of scale population. On plot 4, 72 percent of the twigs that carried heavy populations of adult females and egg masses flagged, whereas in both the medium and light infestations only 39 percent flagged.

During the 1938 reexamination of the flagging of artificially infested ponderosa pine trees on plot 1, it was discovered that there were heavy scale populations on flagged twigs and much lighter populations on the green infested twigs. A more thoroughly determination of scale-population density on flagged and green twigs was made, therefore, from infestations in the field near Prescott. Since preadult population counts have shown that the largest number of insects occur on the second year's growth, the 2-year-old part of the branch was used as a basis for comparison. A collection of 285 twigs was made at random from infested trees. The twigs were segregated by length of the second-year growth and were examined with the aid of a microscope. The results presented in table 3 indicate (1) that the population density of scale insects on flagged twigs is over twice that on green twigs [7] and (2) that flagging contributed to a high mortality of the *Matsucoccus* scale.

TABLE 3.—*Preadult Matsucoccus Scales Found on Second-Year Growth of Ponderosa Pine Collected at Random Near Prescott, Ariz., in 1938*

	Flagged twigs						Green twigs				
Twigs involved	Second-year growth		Scales			Twigs involved	Second-year growth		Scales		
	Average length	Average diameter	Living	Dead	Average per twig		Average length	Average diameter	Living	Dead	Average per twig
Number	*Inches*	*Inches*	*Number*	*Number*	*Number*	*Number*	*Inches*	*Inches*	*Number*	*Number*	*Number*
20	0.9	0.4	21	366	19.3	28	1.2	0.3	211	67	9.9
20	2.1	.3	41	559	30.0	28	2.0	.2	345	83	15.3
42	3.0	.3	101	1,309	33.6	31	3.0	.3	359	109	15.1
22	4.0	.3	67	732	36.3	12	4.0	.3	185	55	20.0
12	5.0	.5	51	585	53.0	19	5.0	.5	280	75	18.7
15	6.0	.5	43	856	59.9	13	6.0	.5	220	40	20.0
4	7.0	.5	4	368	93.0	4	7.0	.6	57	27	21.0
4	8.0	.5	18	302	80.0	1	8.0	.5	22	4	26.0
1	9.0	.3	1	40	41.0	2	9.0	.4	40	21	30.5
3	10.0	.8	20	185	68.3	4	10.0	.7	98	30	32.0
143	----	----	367	5,302	39.6	142	----	---	1,817	511	16.4

[7] The scale populations on flagged twigs are undoubtedly higher than the figures show, since a large number of needles, and consequently of insects, were lost in handling. For example, a study of 57 flagged and 62 green twigs (second year's growth) showed that only 27 percent of the needles on the flagged twigs remained, as compared with 80 percent on the green twigs.

Even though it had been demonstrated by actual counts that a large population of the *Matsucoccus* scale was always present on flagged branches, there was still the question whether killing lesions developed only on the parts of the stem where scales were present.   Therefore, it was deemed advisable to restrict the scales to a specific year's growth by applying barriers of a commercial tree tanglefoot before introducing the scales.   Two types of barrier were used, one consisting of a thin layer of the tanglefoot smeared directly on the stem, and the other of an absorbent-cotton band wrapped around the stem, over which was placed a strip of ½-inch crepe-paper masking tape (fig. 8, *A*). Tanglefoot was then applied to the tape to prevent it from coming in contact with the bark surface and possibly causing injury to the twig.

The barriers were placed at the extremities of a year's growth and were generally effective in restricting the scales, although in a few instances some of the insects escaped (fig. 8, *B*).   Scales were introduced between the barriers by placing egg masses in small, thimble-shaped, copper-screen baskets, which were securely held to the stem with fine copper wire.

Thirty-six trees, 17 on plot 2 and 19 on plot 3, were artificially infested in this manner.   Of these trees 22 had barriers on the second year's growth (fig. 9) and 14 had them on the third year's growth. The results of the infestations are given in table 4, which shows that 34 and 35 percent of the twigs with scale restrictions on the second-year growth flagged, whereas flagging occurred in only 14 and 23 percent of the third-year-growth restrictions.   In nature, as has already been stated, girdling lesions tend to be much less numerous on the second year's growth than on that of the third and fourth years. The girdling lesions occurred only within that part of a branch where scales were restricted, and in no case did killing extend beyond the proximal barrier toward the main stem (fig. 8, *B*).   General observations on many infested twigs where few or no scales became established and which showed no flagging, indicated that neither the barriers nor the copper baskets were alone responsible for the development of girdling lesions.

TABLE 4.—*Artificial infestation of 2- and 3-year-old growth of ponderosa pine twigs with Matsucoccus scales, in plots 2 and 3, Prescott National Forest in 1938*

Plot No.	Part of branch used (age of wood)	Trees			Twigs	
		Involved	Average height	Average diameter breast high	Infested [1]	Flagged [2]
	Years	*Number*	*Feet*	*Inches*	*Number*	*Percent*
2	2	10	12.5	2.6	206	35
	3	7	16.1	5.1	109	14
3	2	12	11.7	3.1	522	34
	3	7	10.5	3.9	238	23

[1] The artificial-infestation experiments in restricting the scale to certain year's growth of the stem were conducted from April 28 to May 13, on plot 2, and from May 9, to 12, on plot 3.
[2] Flag readings were taken on July 24–26, 1939, on plot 2, and on June 26, 1939, on plot 3.
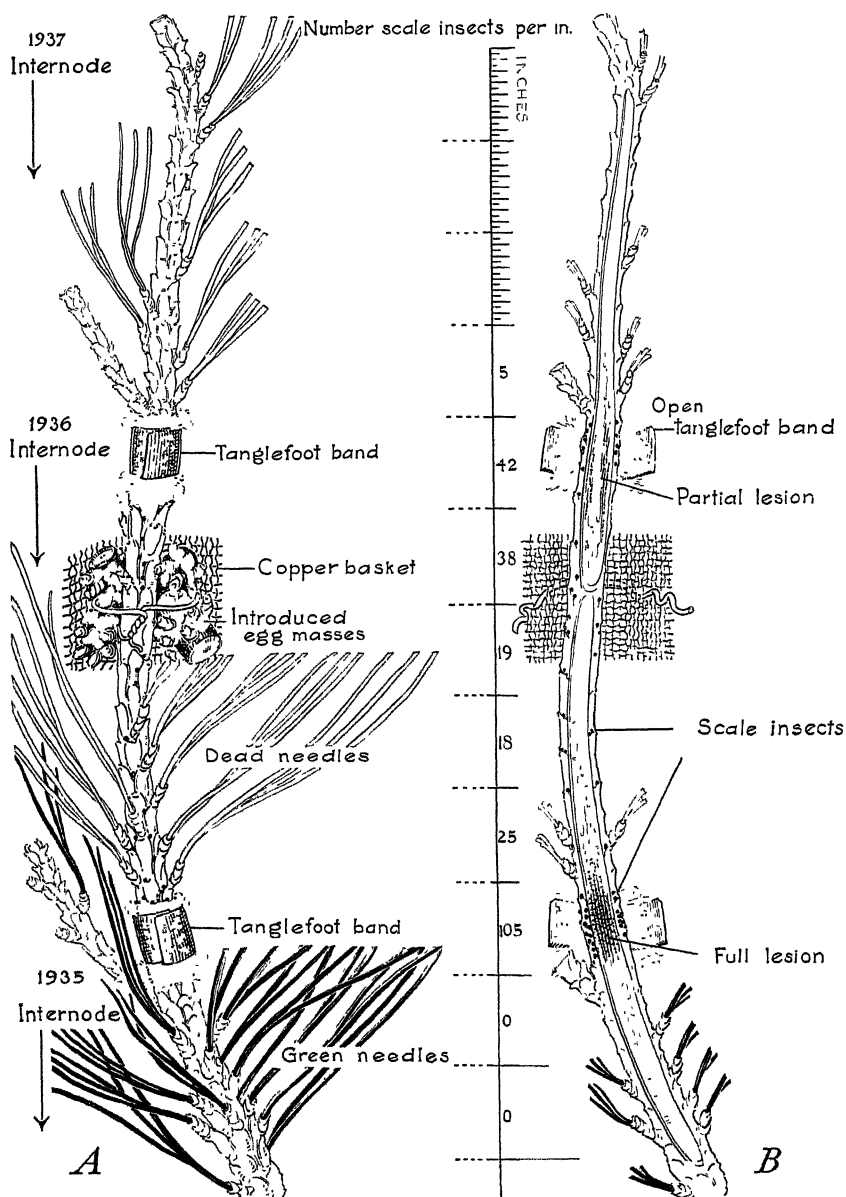
FIGURE 8.—Cotton-tape bands used to restrict *Matsucoccus vexillorum* scales to certain year's growth on the pine branches. *A,* The scale insects were introduced between the barriers by placing egg masses in copper-screen baskets, which were held securely to the stem with fine copper wire. *B,* The scale insects successfully established on test twigs were counted approximately a year after initial artificial introduction. The twigs were sliced with a pocketknife to ascertain killing lesions. There appeared to be a definite correlation between scale population and such lesions.

During the summer of 1939, 60 of the 1,075 branches originally infested with scales were cut and examined in the laboratory. The copper baskets and barriers were removed, and the needles were cut off rather short to permit observation of the insects with the low-power binocular microscope. The branch sections were diagramed
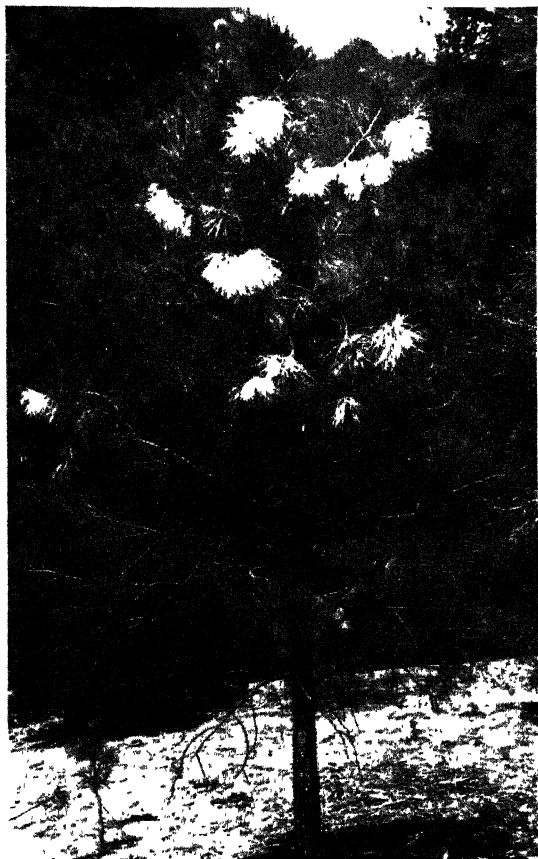
FIGURE 9.—Experimental tree No. 37, on plot 3 in Copper Basin Divide, near Prescott, Ariz., which had barriers applied to the second year's (1937) growth only. Fifty-two percent of the infested twigs flagged from lesions in the second year's growth.

to actual size on drawing paper and measured off into 1-inch intervals. The number of preadults found on each inch of stem was recorded in the corresponding interval on the diagram. After the counts were completed, the stems were carefully sliced with a pocketknife, to determine the exact position of the lesions (fig. 8, *B*).

When used alone, the tape barriers were more effective than the tanglefoot. The scale larvae apparently preferred to settle under the cotton bands, because these afforded them some protection. As many as 225 preadults have been observed under one band. The results of the scale counts on the 60 twigs with barriers indicate that, when there is

an average population density of 100 or more preadult scales of the same generation per linear inch of stem with a diameter of ½ to ¾ inch, killing lesions will be associated with these insects.

PATHOLOGICAL ANALYSIS OF ARTIFICIALLY INFESTED TWIGS

The 21 artificially infested trees on plot 2 that were reserved•for pathological studies by the Bureau of Plant Industry had a total of 483 artificially infested twigs. On 294 of them the insects were not restricted, on 119 they were restricted to the 3-year-old (1936) growth, and on 70 they were restricted to the 2-year-old (1937) growth, both types of barriers previously described having been used. These three classes of restrictions will hereafter be referred to as series A, B, and C. Five approximately equal random collections from these trees were made as follows: (1) In December 1938, (2) in January 1939, (3) in March 1939, (4) in April 1939, and (5) in May 1939.

SCALE POPULATION

The twigs were examined to ascertain whether the scales had become established. This was determined by the presence or absence of preadults on twigs of collections 1 to 4, and by presence or absence of egg masses or preadult "cases" on twigs of collection 5, since the preadults had developed into adult females by May. In collections 1 to 4 an estimate of scale intensity on each year's growth was obtained on the basis of preadult population. The fifth collection was considered unreliable for this purpose. A record was also kept of instances in which it could be determined with certainty that the preadults located within the bounds of a lesion were dead.

LESIONS

A record was kept of the number and size of lesions found on each year's growth. Lesions ranged in size from small but readily visible necrotic spots in the bark at the base of needles to diffuse necrosis extending over two or more years' growth. In large lesions, covering several years' growth, the probable age of the stem portion on which they originated, as determined by resin infiltration and general necrotic appearance, was recorded.

FUNGI

Cultures were made from all lesioned twigs in collections 1 to 4. The twigs were surface-sterilized with 1:1,000 mercuric chloride, the outer bark was removed, and small chips of necrotic tissue were planted on malt-agar plates. The number and type of fungus colonies developing from the chips were then recorded.

• For all five collections, records were also kept of all lesioned twigs bearing fruiting bodies of *Cenangium* sp., either visible on the surface or beneath the bark. During the period of the first four collections (December to April) there appeared to be no significant increase in the average size or number of lesions, nearly all of which occurred on the 3- and 4-year-old growth. The fifth collection (May) showed in addition an abundance of small lesions on the 2-year-old growth. No explanation can be offered at this time for the sudden and late appearance of lesions on the 1937 growth.

GENERAL RESULTS

The general results obtained in series A, B, and C are shown in table 5. No lesions were found on twigs on which scales had not become established.

TABLE 5.—*Pathological Analyses of 21 Artificially Infested Trees From Collections 1 to 5, Plot 2, Made December 1938 to May 1939, at Prescott, Ariz.*

Examinations	Series A	Series B	Series C
	Number	*Number*	*Number*
Twigs artificially infested	294	119	70
Twigs upon which scales became established	211	94	43
Lesioned twigs with scales	109	67	16
Lesioned twigs that flagged	29	1	1
Lesioned twigs that probably would flag	32	16	1
Twigs with small lesions only	48	50	11

The percentage of lesioned twigs that flagged in the artificial-infestation studies was markedly higher than seems to be the case in nature. Presumably this was due to the fact that the artificially infested twigs, in general, carried greater scale populations than would infested twigs in a sample taken at random in nature. Most of the experimentally infested twigs with only small lesions would probably have lived.

In collections 1 to 4 (December to April) fruiting bodies of *Cenangium* were present on 36 percent of the lesioned twigs. In 78 percent of the lesioned twigs there were dead scales over lesioned tissue.

These analyses indicate that scales are probably the primary cause of lesion formation, since lesions occurred only on twigs where scales were present. It is also evident that some infested twigs do not develop lesions. In series A 52 percent and in series B 71 percent of the twigs on which scales were present developed lesions. The data for series C are not considered comparable, because practically no lesions were found until May.

TABLE 6.—*Fungi Cultured From 21 Artificially Infested Trees, Collections 1 to 4, Plot 2, December 1938 to April 1939, at Prescott, Ariz.*

Series	Twigs cultured			Twigs producing—		
	Total	Produc-ing fungi	Sterile	Cenangi-um sp.	Sapro-phytes [1]	Other fungi
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
A	68	85	15	71	34	7
B	26	85	15	62	42	8
C	2	100	0	[2] 50	50	0
Total	96	85	15	68	36	7

[1] Black, fast-growing, ubiquitous saprophyte.
[2] Lesion occurred on 1935 growth.

Table 6 gives data on the fungi cultured from samples taken from collections 1 to 4. *Cenangium* sp. is the fungus most frequently associated with blight lesions at all stages of their development.

Analysis of the artificially infested twigs in series A showed that lesions originated only where scales were present. On 164 twigs 4 or

more years of age on which scales became established, infestations were found on 152 2-year-old segments, 138 3-year-old segments, and 71 4-year-old segments. No lesions originated in infested growth of 1937 (2 years old) until the May collection, 33 originated in infested growth of 1936, and 38 in infested growth of 1935. This suggests that, although the heaviest population of scales is usually on the 2-year-old growth, it is possible that the mere presence or absence of scales on the 3- or 4-year-old growth may be a more important factor in the formation of lesions than is scale-population density here or elsewhere on the twig.

ASSOCIATED FUNGI

The fungus most frequently associated with the so-called "twig blight" is a species of *Cenangium* that is morphologically and physiologically similar to *C. ferruginosum* Fr. (*C. abietis* Pers. ex Rehm.). Duby. Long [8] considered this fungus to be an important and beneficial agent in the self-pruning of ponderosa pine. *Cenangium* sp. is often isolated from lesions, and it invariably fruits abundantly on flagged twigs. It is not uncommon to find the black stromata of this fungus beneath the bark of lesioned twigs that probably would have flagged although the needles were still green and fairly succulent. This suggests that the fungus becomes established well in advance of the death of the twig. Between November 1934 and March 1938 it was isolated from 31 percent of 430 incipient nongirdling lesions cultured, and from 54 percent of 458 flags. In the different lots studied during this period these percentages ranged from 8 to 57 percent of the lesions and from 35 to 98 percent of the flags.

Schwartz [9] attributed a twig-dying of pine in Europe to *Cenangium abietis*, but did not verify his contention with inoculation experiments. More recently Liese [10] stated that the European disease, which is similar in some respects to the twig flagging herein described, results from the combined effects of a gall midge (*Cecidomyia brachyptera*) and a parasitic fungus. By association, *Cenangium abietis* appeared to be the most likely fungus, although neither its connection with the midge nor its parasitic nature had been positively established.

A small and inadequate amount of isolation work from various stages of *Matsucoccus vexillorum* from Arizona has failed to indicate that it is a vector of a micro-organism.

In addition to *Cenangium* sp., 10 other fungi and several bacteria have been isolated from lesioned twigs. None of these has been found as frequently as *Cenangium*, nor have inoculations demonstrated that they are involved in the twig killing. Of the fungi, one tentatively identified from its imperfect stage as *Scoleconectria* sp. exhibited parasitic tendencies to a somewhat greater extent than the others. It was rather infrequently isolated from recently lesioned

---

[8] LONG, W. H. THE SELF PRUNING OF WESTERN YELLOW PINE. Phytopathology 14: 336–337. 1924.
[9] SCHWARTZ, F. DIE ERKRANKUNG DER KIEFERN DURCH CENANGIUM ABIETIS. 126 pp., illus. Jena, 1895.
[10] LIESE, J. ZUM TRIEBSTERBEN DER KIEFER. Deut. Forstwirt. 17: 381–383. 1935.

tissue, and hundreds of inoculations with it failed to produce flags, except where the technique was so drastic that most of the checks were killed.

SUMMARY AND CONCLUSIONS

Research on the so-called "twig blight" of ponderosa pine in the Southwest indicates that the flags and lesions characteristic of the trouble occur only on twigs that have been attacked by the scale insect *Matsucoccus vexillorum* Morrison. This scale is therefore believed to be the primary cause of the blight. Although heavy flagging appears only in areas where the insect is abundant, infested twigs frequently develop no lesions.

The intensity of flagging in areas infested with *Matsucoccus* varies widely from year to year. The epidemic conditions which existed in 1933 and 1934 have been followed by 12 years of relatively low, though somewhat fluctuating, intensity. Since scale mortality was much higher on "flags" than on green infested twigs, it is logical to assume that during epidemic years the scale population of the area becomes drastically reduced, and several seasons may be necessary for it to build up to the point where damage to the pine again becomes conspicuous.

Damage to the forest by the blight was found to be lighter than was at first feared. Tree mortality has been insignificant and has occurred primarily among seedlings and saplings. Since the forest types affected by the trouble are well stocked and are valued primarily for watershed protection or recreational use rather than timber production the damage is of no great economic importance. There is no indication that this twig blight will in any way endanger the pine forests of the United States.

Eleven fungi and several bacteria occur rather frequently in flagged twigs or in lesioned tissue of living twigs. A fungus morphologically similar to *Cenangium ferruginosum* is the one most commonly associated with lesioned tissue, and it almost invariably fruits on flagged twigs. Inoculations with these fungi and bacteria indicate that none of them are sufficiently parasitic to cause flagging independently. The lesions seem to originate in the phloem at fascicle traces, a condition that suggests close association with the scale, since the pre-adults usually settle in the axils of the needle fascicles. Many lesions are arrested by the action of a pathological cork cambium which walls off the necrotic tissue before a lethal girdle can be effected.

INHERITANCE OF WEIGHT PER UNIT LENGTH OF CULM AND OTHER CHARACTERS IN KANRED × COPPEI WHEAT [1]

By I. M. ATKINS [2]

Agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

Lodging of the cereal crops has long been a subject of study. On land high in fertility or in seasons of abundant precipitation, it may often become a serious factor in the harvesting of the crop and may cause heavy financial loss to the grower. With the advent of the small combine-harvester-thresher in the more humid areas where cereals are grown, the problem becomes increasingly important.

Mechanical devices of various types have been invented to test lodging resistance of small grains in the laboratory and thus aid in selecting strains resistant to lodging. Some of these devices have been found satisfactory, while others have been reported as unsatisfactory. At Texas Substation No. 6, Denton, Tex., weight per unit length of culm near the base of the plant (herein referred to simply as weight per unit length) has been successfully used for a number of years to test the relative resistance to lodging of varieties and strains of winter wheat. Extensive studies of varieties under field conditions show that this measure is significantly correlated with lodging in the field. The method has been found less satisfactory for oats and barley.

The present study is concerned with measurements of weight per unit length, of breaking strength of the culm near the base of the plant (herein referred to simply as breaking strength), and of other morphological characters in plants of the early segregating generations of a hybrid between Kanred, a lodging-susceptible common hard red winter wheat (*Triticum aestivum* L. (*T. vulgare* Vill.)) having low weight per unit length, and Coppei, a lodging-resistant club wheat (*T. compactum* Host) having high weight per unit length. The purpose was to study the variability and inheritance of these characters and to determine to what extent measurements of them in early generations might serve as an aid in isolating lines that maintain high weight

per unit length or other desired characteristics in later generations. The plan was to determine the correlation between morphological characters and lodging under natural conditions in the field; but, as no lodging occurred during the period of study, this phase of the experiment could not be carried out. As the parent varieties Kanred and Coppei differed in respect to presence of awns, pubescence of glumes, and spike length, the inheritance of these characters was studied in the same material.

REVIEW OF LITERATURE

The literature on lodging of small grains is extensive. Rather complete reviews were made by Welton and Morris (*14*),[3] the Imperial Bureau of Plant Genetics, School of Agriculture,[4] and Phillips, Davidson, and Weihe (*8*). The papers reviewed pertained largely to causes of lodging, relation of morphological characters to lodging, and mechanical tests for measuring resistance to lodging. No reports on the inheritance of lodging resistance in wheat as determined mechanically were found. Such methods were used, however, by Smith (*11*) on oats, by Bose, Aziz, and Bhatnagar (*3*) on barley, and by Ramiah and Dharmalingam (*9*) on rice. Heritable differences in lodging resistance have been recognized by many workers.

Basing their distinctions upon visual observations and evaluations of hybrid progenies of wheat crosses, several workers have shown that strength of straw is an inherited character. Clark, Florell, and Hooker (*4*) found an association between awnedness and tendency to lodge. Goulden and Neatby (*5*) found a genetic linkage between weak straw and mature-plant resistance to stem rust. Kilduff (*7*) studied the tendency to lodge in two crosses on Kota wheat. He found in one a significant correlation between bunt resistance and weak straw and in the other a correlation between bunt resistance and strong straw. Waldron (*13*) also studied lodging resistance in a cross involving the variety Kota. He found that the progeny ranged from strains having weaker straw than Kota to strains about midway between the parents in lodging resistance. Torrie (*12*) classified the F_3 population of two crosses on the variety Caesium on the basis of a lodging index computed from the percentage lodged and the angle of lodging. No genetic interpretation was proposed, but the F_3 lines ranged from the weak-strawed parent to the strong-strawed.

The most extensive use of a mechanical testing device on hybrid populations was made by Smith (*11*), who tested several hundred F_2 plants from an oat cross and compared their reactions with those of their F_3 progenies. He concluded that measurements of the F_2 plants alone are not adequate to determine resistance to lodging. Hamilton (*6*), working in Canada, also found that the weight per unit length is insufficient in itself to test lodging resistance of oat varieties.

MATERIAL AND METHODS

The present study of the inheritance of morphological characters that may be associated with lodging was started in 1932, when the

---

[3] Italic numbers in parentheses refer to Literature Cited, p. 71.
[4] IMPERIAL BUREAU OF PLANT GENETICS. LODGING IN CEREALS. 8 pp. Cambridge. 1931. [Processed.]

first cross was made. Breaking-strength tests were made on varieties of wheat grown in field plots before that time, and the data were used as a guide in selecting parental material.

For the lodging-susceptible parent, the common hard red winter wheat variety Kanred was selected. Kanred, a pure-line selection from the Crimean (Turkey) variety, is of moderate height and has an awned, slender spike with white, glabrous glumes. It tillers abundantly and, except for susceptibility to leaf rust, is well adapted to conditions in north-central Texas.

The lodging-resistant parent selected was Coppei, a club wheat having awnless, compact spikes, pubescent glumes, short culms, and relatively low tillering ability in Texas. It is poorly adapted to Texas because of its high susceptibility to leaf and stem rusts. In the present study, however, these diseases were controlled by dusting with sulfur. In varietal comparisons of weight per unit length in another experiment, the 5-year average weight per unit length of Coppei was 14.3 gm. per five-culm sections compared with an average of 8.4 gm. per five-culm sections for Kanred.

All tests were made on single plants grown in 10-foot nursery rows spaced 12 inches apart. Plants were spaced accurately 3 inches apart in the rows by dropping the seed through holes in a board. After the plants emerged, missing hills were replanted with a variety that could be recognized and discarded at maturity. At harvesttime plants surrounded by perfect stands were pulled and placed under cover for future testing.

The characters studied in all or some of the seasons included breaking strength, weight per unit length, total weight of culm, plant height, diameter of culm near the base of the plant, length of lower internode, weight of head, length of head, head type, awn type, and pubescence of glume. Tests and measurements of these characters were made as follows: Culms were cut off at the ground level; the tallest culm was measured for plant height, length of internode, and head length; and data were then recorded on head type, awn type, and presence of pubescence of glumes. Heads were then removed from all culms and five selected at random were weighed; culms were then weighed in units of five for the weight of culm.

In 1934, 1936, and 1937 the tests of breaking strength were made by means of the Salmon (*10*) breaking-strength-of-straw machine; again the unit sample was five culms. When possible, two or more tests per plant were made and the results were averaged.

Weight per unit length was obtained by cutting sections of culm 10 cm. long from the region of the first straight internode. Such a section included one node and parts of two internodes. Sections were cut from five culms at one time by means of the cutter shown in figure 1. These sections were weighed on a scale sensitive to 0.01 gm. Two or more tests were made on each plant having sufficient tillers.

After the culm sections were weighed, diameter of culm was determined by placing the five sections side by side and measuring the overall diameter with a vernier caliper.

Because of the large seasonal influence on morphological characters, it was considered desirable to grow as many generations as possible each season. The cross was remade each year in order to have several generations available for study. The 1934 plantings consisted of the
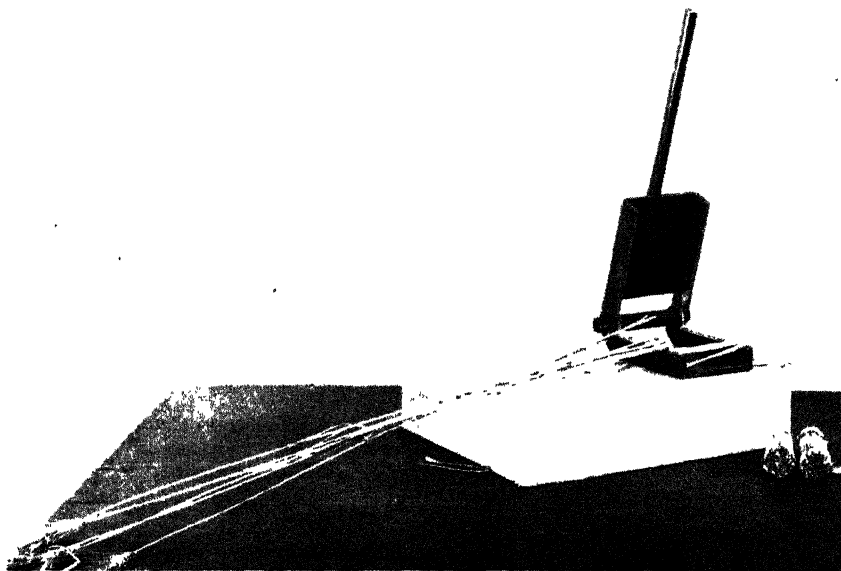
FIGURE 1.—Straw cutter used to cut 10-cm. sections of culms for determination of weight per unit length.

parents and the F_1 and F_2 populations of the hybrid. All plantings were winterkilled in 1935. The 1936 material consisted of parents, F_1 and F_2 populations, and F_3 progeny rows. Twenty plants selected at random were tested from each of the progeny rows. Parents and F_4 progeny rows were grown and tested in 1937. The F_3 plants selected for planting the F_4 progeny rows included extremes as well as random selections.

Because of inadequate parental material for comparison with hybrids in the early tests and unfavorable seasonal conditions, the test was redesigned on a more extensive scale in 1940. Seasonal conditions were favorable, and normal plants were produced. Four hundred and eighty plants from the F_2 populations, 31 F_1 plants, and 200 plants from each of the parent populations were selected at random for testing. In 1941 the tests consisted of duplicate progeny rows of 100 F_3 lines. Ten progeny lines from each parent were grown for comparison. Twenty randomly selected plants were tested from each duplicate row. Excessive precipitation throughout the 1941 season was unfavorable for the development of the plants.

The 1942 plantings were destroyed by green bugs. In 1943, 128 F_4 lines were grown in duplicate progeny rows along with 25 lines from each parent for comparison. Twenty plants selected at random were tested from each row. Seed for planting the F_4 was from plants within F_3 lines representing the extremes in weight per unit length and in variability. Low temperatures during the winter of 1943–44 damaged the planting, killing all progeny rows of the Coppei parent and many hybrid progeny rows. Only 83 lines could be tested, and both replications were available for only 39 lines.

EXPERIMENTAL RESULTS

VARIABILITY OF MORPHOLOGICAL CHARACTERS

Because of the importance of environmental factors it was desirable to determine their influence on the characters being studied. This can be approximated by comparing the coefficients of variability of the parental and F_1 plants, in which all the variability should be environmental as no genetic segregation is expected, with those of the F_2 population grown under the same conditions, in which both genetic segregation and environment play a part. No special planting was made for this comparison; but, as the selections planted for the study of inheritance of weight per unit length were made without regard for other characters, the population may be considered random. Such data for the 1934, 1936, and 1940 seasons are given in table 1.

TABLE 1.—*Variability of certain plant characters of parents and F_1 and F_2 populations of the wheat cross Kanred × Coppei, Denton, Tex., 1934, 1936, and 1940*

Type of material and parent or cross	Year	Coefficient of variability for—							
		Breaking strength	Weight per unit length	Weight of 5 culms	Diameter of 5 culms	Plant height	Length of lower internode	Weight of 5 heads	Head length
Homogeneous:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Kanred parent_____	1934	16 19	13. 02	14 33	5. 96	5 47	14. 37	18. 07	10 53
Do_____	1936	8. 68	7 43	11. 21	5 78	4 77	16. 75	4. 58	9. 63
Do_____	1940	_____	12. 91	18. 24	8. 11	4. 78	10 34	18 55	15.37
Coppei parent_____	1934	10 61	9. 00	19 55	6. 95	7 44	12. 68	20. 68	9. 44
Do_____	1936	1. 35	7. 72	12 38	5. 81	5. 63	8. 63	13. 73	5. 66
Do_____	1940	_____	9. 86	14. 11	7. 27	4. 88	16. 79	15. 11	10. 61
Kanred × Coppei F_1__	1934	13. 31	11. 47	11. 77	5. 39	4. 44	11. 83	15. 77	9. 49
Do_____	1936	. 34	6 62	16. 19	4. 18	4 27	15. 41	16. 48	6. 37
Do_____	1940	_____	9. 10	9. 96	4 37	3 88	18. 22	13. 19	9. 83
Mean_____	_____	8 41	9. 68	14 19	5. 98	5. 06	13. 89	15. 13	9. 66
Heterogeneous:									
Kanred × Coppei F_2____	1934	14. 82	19 18	20 18	8. 67	8. 74	16. 42	26. 65	25. 58
Do_____	1936	11. 63	12. 60	11. 65	5 07	7. 42	16 89	15. 10	36. 90
Do_____	1940	_____	16. 16	18. 47	8. 69	8. 46	20. 02	21. 14	34. 52
Mean_____	_____	13. 23	15. 98	16 77	7. 48	8. 21	17. 78	20. 96	32. 33

The variability of characters, as measured by the coefficient of variability, is of approximately the same order in both homogeneous and heterogeneous material (table 1). The least variable character in most classes was plant height followed closely by diameter of culm. In the homogeneous material weight of head was the most variable character, followed closely by weight of culm and length of lower internode. All characters were more variable in the F_2 as would be expected. Because of the wide difference in head types of the parents, head length and weight of heads were highly variable in the F_2 populations. The data indicate that one-half to three-fourths of the total variability of the heterogeneous material may be due to environmental influences.

VARIABILITY AND INHERITANCE OF WEIGHT PER UNIT LENGTH

In order to determine whether the parental varieties were pure for the characters under investigation and what variability was to be

expected in uniformly spaced plant material, random selections and others representing the extremes in weight per unit length of each parent were grown and tested under the same conditions as the hybrid populations each season. The data for parents and early hybrid generations for 1934, 1936, and 1940 are given in table 2. As none of these tests could be replicated, the data are presented in convenient, arbitrary intervals in the table.

It is evident from the data that the parents are statistically different in mean weight per unit length, although the ranges overlapped in 1940. The variability of the parents was high in some seasons; but this appears to be environmental, since the progeny lines grown from plants having widely different values were rather uniform the following season. It appears, therefore, that the parents were essentially homozygous for the characters studied. · The F_1 plants, while intermediate between the parents each season, tended to approach the Coppei parent in mean weight per unit length. The range of weight per unit length for the F_2 was much greater than for the F_1 or parental material, as would be expected.

The results of tests of the F_3 progeny lines in comparison with parent progeny lines in 1936 and 1941 are given in table 3. The lines are grouped by weight per unit length and coefficient of variability of weight per unit length with class centers based on the standard error of a difference between two lines. The data show that a majority of the F_3 lines were intermediate between the parents but that the range of F_3 lines recovered was from the lowest Kanred lines to well within the range of the Coppei parent. The recovery of F_3 lines having a wide range of weight per unit length indicates that inheritance of this character involves multiple factors. In both seasons F_3 lines approaching homozygosity, as indicated by low coefficient-of-variability values, were recovered in a wide range of weight-per-unit-length classes.

The analysis of variance of the F_3 progeny lines grown in two replications in randomized blocks in 1941 is given in table 4. The least significant difference between two strains at the 5-percent level was 0.105 gm. The differences between progeny lines are highly significant.

Correlation coefficients were calculated between the weight per unit length of F_2 plants of 1934 and the mean of their F_3 progeny lines grown in 1936 and between the F_2 plants of 1940 and the mean of their F_3 progeny lines grown in duplicate rows in 1941. These coefficients were 0.414 and 0.609, respectively. Both values exceed the 1-percent level and show that F_2 plants may be expected to transmit the characteristics of their weight per unit length to a fair proportion of their progeny lines. The relation of the two generations in the more accurate test of duplicate progeny rows in 1941 is shown in a scatter diagram (fig. 2).

Tests of selected F_4 progenies were made in 1937 and 1943, the latter in duplicate plantings. Data for these are given in table 5. As in table 3, the data are grouped by weight per unit length and coefficient of variability of weight per unit length with class centers based on the standard error of a difference between two classes in the 1943 test. The 1937 data are included in the same classes for con-

TABLE 2.—*Frequency distribution for weight per unit length of plants of parents and F_1 and F_2 populations of the wheat cross Kanred × Coppei, Denton, Tex., 1934, 1936, and 1940*

Year and parent or cross	Plants with indicated weight per unit length (grams) per 5-culm sections												Total plants	Mean	Standard deviation	Coefficient of variability
	0.31–0.40	0.41–0.50	0.51–0.60	0.61–0.70	0.71–0.80	0.81–0.90	0.91–1.00	1.01–1.10	1.11–1.20	1.21–1.30	1.31–1.40	1.41–1.50				
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Grams*	*Gram*	*Percent*
1934																
Kanred parent	13	7	0	0	0	0	0	0	0	0	0	0	20	0.430	0.056	13.02
Coppei parent	0	0	0	6	6	8	0	0	0	0	0	0	20	.778	.070	9.00
Kanred × Coppei F_1	0	0	13	6	7	0	0	0	0	0	0	0	26	.654	.075	11.47
Kanred × Coppei F_2	4	30	47	46	30	13	3	0	0	0	0	0	173	.656	.126	19.18
1936																
Kanred parent	0	0	0	5	13	7	0	0	0	0	0	0	25	.751	.056	7.13
Coppei parent	0	0	0	0	0	0	1	8	6	6	0	0	21	1.138	.088	7.72
Kanred × Coppei F_1	0	0	0	0	0	0	2	1	2	0	0	0	5	1.056	.070	6.62
Kanred × Coppei F_2	0	0	1	8	37	51	54	33	1	0	0	0	185	.897	.113	12.60
1940																
Kanred parent	0	0	3	25	72	71	22	7	0	0	0	0	200	.805	.104	12.91
Coppei parent	0	0	0	0	0	5	25	55	64	39	10	2	200	1.126	.111	9.86
Kanred × Coppei F_1	0	0	0	0	0	1	5	10	11	4	0	0	31	1.088	.099	9.10
Kanred × Coppei F_2	0	3	19	60	124	144	84	31	15	0	0	0	480	.829	.134	16.16

TABLE 3.—*Frequency distribution for weight per unit length grouped by coefficient-of-variability classes for parental and F_3 progeny lines of the wheat cross Kanred × Coppei, Denton, Tex., 1936 and 1941*

Year and parent or cross	Coefficient-of-variability class (Percent)	Lines with indicated weight per unit length (grams) per 5-culm sections											Total lines
		0.567–0.619	0.620–0.672	0.673–0.725	0.726–0.778	0.779–0.831	0.832–0.884	0.885–0.937	0.938–0.990	0.991–1.043	1.044–1.096	1.097–1.149	Number
		Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
1936													
Kanred parent	5.1– 8.5	0	0	0	1	0	0	0	0	0	0	0	1
Coppei parent	5.1– 8.5	0	0	0	0	0	0	0	0	0	0	1	1
Kanred × Coppei F_3	5.1– 8.5	0	0	0	0	2	1	3	0	0	0	0	6
	8.6–12.0	0	0	6	13	27	15	9	5	2	0	0	77
	12.1–15.5	0	0	3	11	19	17	6	3	0	0	0	59
	15.6–19.0	0	0	0	2	3	1	2	0	0	0	0	8
	19.1–22.5	0	0	0	2	0	0	0	0	0	0	0	2
	22.6–26.0	0	0	0	0	0	0	1	0	0	0	0	1
Total		0	0	9	28	51	34	21	8	2	0	0	153
1941													
Kanred parent	5.1– 8.5	0	3	0	0	0	0	0	0	0	0	0	3
	8.6–12.0	4	1	0	0	0	0	0	0	0	0	0	5
	12.1–15.5	2	0	0	0	0	0	0	0	0	0	0	2
Total		6	4	0	0	0	0	0	0	0	0	0	10
Coppei parent	5.1– 8.5	0	0	0	0	0	0	0	0	0	1	0	1
	8.6–12.0	0	0	0	0	0	0	0	0	0	3	2	5
	12.1–15.5	0	0	0	0	0	0	0	1	2	0	1	4
Total		0	0	0	0	0	0	0	1	2	4	3	10
Kanred × Coppei F_3	5.1– 8.5	0	0	1	0	0	0	0	0	0	0	0	1
	8.6–12.0	0	1	4	1	8	4	1	1	0	0	0	20
	12.1–15.5	1	0	4	6	15	8	8	3	1	0	0	46
	15.6–19.0	0	0	2	4	7	2	2	0	1	0	0	18
	19.1–22.5	0	0	1	0	0	0	0	0	0	0	0	1
	22.6–26.0	0	0	0	0	1	0	0	0	0	0	0	1
Total		1	1	12	11	31	14	11	4	2	0	0	87

TABLE 4.—*Analysis of variance of F_3 progeny lines of Kanred × Coppei wheat grown in duplicate randomized blocks, Denton, Tex., 1941*

Source of variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	F obtained	F required at 5-per-cent level
Blocks	1	0.00406	0.00406		1.424	3.96
Progenies	86	1.0915	.01269		4.452	1.38
Error	86	.24484	.00285	0 053		
Total	173	1.34040				
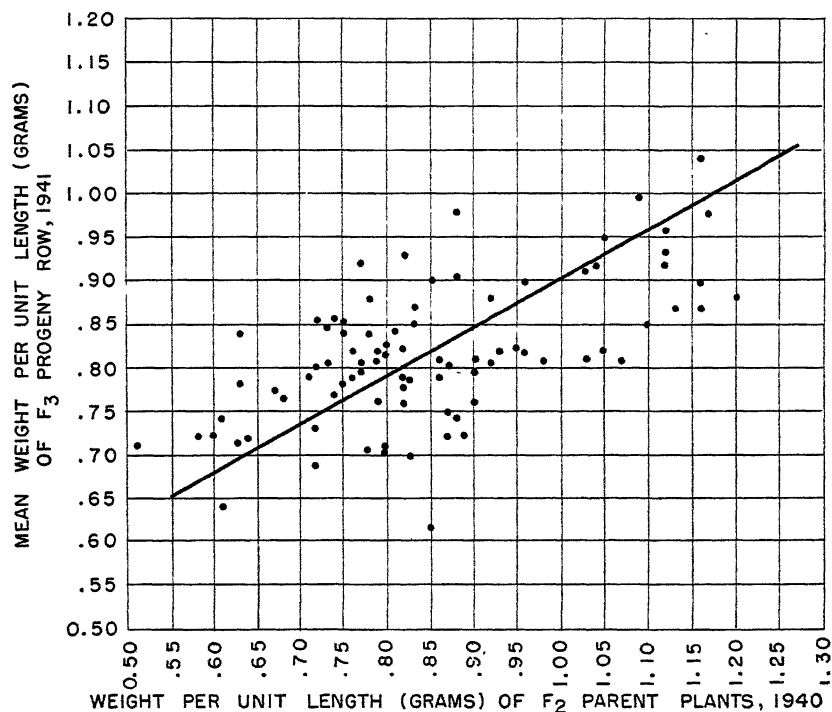
FIGURE 2.—Relation of weights per unit length of Kanred × Coppei F_2 wheat plants grown in 1940 and the means of their F_3 progeny lines grown in 1941, Denton, Tex.

venience. Low temperatures during the winter of 1943 killed all Coppei lines and many hybrid progeny lines as well, so that only 83 could be tested. Of these, both replications were available for only 39 lines.

The analysis of variance for the 39 lines in duplicate plantings is given in table 6. The calculated least significant difference at the 5-percent level was 0.100 gm. per 5-culm sections. The differences between progeny lines were again highly significant.

TABLE 5.—*Frequency distribution for weight per unit length grouped by coefficient-of-variability classes for parental and F₄ progeny lines of the wheat cross Kanred × Coppei, Denton, Tex., 1937 and 1943*

Year and parent or cross	Coefficient-of-variability class (Percent)	Lines with indicated weight per unit length (grams) per 5-culm sections											Total lines
		0.371–0.420	0.421–0.470	0.471–0.520	0.521–0.570	0.571–0.620	0.621–0.670	0.671–0.720	0.721–0.770	0.771–0.820	0.821–0.870	0.871–0.920	
1937													
Kanred parent	6.5–10.3	0	0	0	1			0	0	0	0	0	1
	10.4–14.1	0	0	0	2	2	1	0	0	0	0	0	5
Total		0	0	0	3	2	1	0	0	0	0	0	6
Coppei parent	6.5–10.3	0	0	0	0	0	0	0	0	0	0	2	2
	10.4–14.1	0	0	0	0	0	0	0	0	1	0	1	2
	14.2–17.9	0	0	0	0	0	0	0	0	1	0	0	1
Total		0	0	0	0	0	0	0	0	2	0	3	5
Kanred × Coppei F₄	6.5–10.3	0	0	1	0	2	6	10	4	5	1	0	29
	10.4–14.1	0	0	0	1	3	24	18	13	5	1	0	65
	14.2–17.9	0	0	0	0	3	2	3	4	1	2	0	15
Total		0	0	1	1	8	32	31	21	11	4	0	109
1943													
Kanred parent	6.5–10.3	0	0	0	4	2	0	0	0	0	0	0	6
	10.4–14.1	0	0	0	4	3	0	0	0	0	0	0	7
Total		0	0	0	8	5	0	0	0	0	0	0	13
Coppei parent [1]		---	---	---	---	---	---	---	---	---	---	---	---
Kanred × Coppei F₄	6.5–10.3	1	0	0	0	0	0	2	1	1	1	1	7
	10.4–14.1	0	1	1	4	7	7	6	3	5	4	3	40
	14.2–17.9	1	1	1	4	3	4	3	3	2	0	0	21
	18.0–21.7	0	2	1	2	1	0	2	0	0	0	0	9
	21.8–25.5	0	1	1	0	0	1	0	0	0	0	0	3
	25.6–29.3	1	2	0	0	0	0	0	0	0	0	0	2
	29.4–33.1	0	1	0	0	0	0	0	0	0	0	0	1
Total		3	7	3	10	11	12	13	7	8	5	4	83

[1] Winterkilled.

TABLE 6.—*Analysis of variance of F_4 progeny lines of Kanred × Coppei wheat grown in duplicate randomized blocks, Denton, Tex., 1943*

Source of variation	Degrees of freedom	Sum of squares	Mean square	Standard deviation	F obtained	F required at 5-percent level
Blocks_____	1	0.019225	0 019225	_____	7.91	4.10
Progenies_____	38	1.261162	.033188	_____	13.66	1.71
Error_____	38	.092319	.002429	0.049	_____	_____
Total_____	77	1.372706	_____	_____	_____	_____

The data presented in table 5, although somewhat unsatisfactory because of the small numbers, appear to confirm the conclusions reached from studies of the earlier generations. In 1943, 13 of the 83 hybrid lines had a lower weight per unit length than any of the Kanred lines and 34 had a mean weight per unit length within or below the range of the Kanred parent. This tendency of a larger number of hybrid lines to approach the lodging-susceptible parent occurred in other seasons with other hybrid material. It also was observed in crosses of spring wheat by Waldron (*13*) and by Kilduff (*7*).

The progeny lines of low variability probably are approaching homozygosity for weight per unit length. Of the 31 F_4 progeny lines grown in 1943 from the more uniform F_3 lines, the majority were no more variable than the Kanred parent. The correlation coefficient between weight per unit length of the F_3 plants and their F_4 progeny lines was 0.569. Similar coefficients for all F_4 lines tested in 1937 and 1943 were 0.526 and 0.623, respectively. All three are highly significant statistically and indicate that tests on F_3 plants of hybrid populations should aid in selecting lines of high weight per unit length and probably also for lodging resistance. The relation of weights per unit length of F_3 plants and the means of their F_4 progeny lines grown in 1943 is shown in figure 3.

VARIABILITY AND INHERITANCE OF BREAKING STRENGTH

In 1934, 1936, and 1937 the breaking-strength determinations on all material were made with the Salmon (*10*) machine. When this study was first undertaken the close relation between breaking strength and weight per unit length was unknown. It soon became apparent (*1, 2*) that the more easily determined measure of weight per unit length could be substituted for breaking-strength tests The close relation between these characters for several hundred varieties that have been studied is indicated by correlation coefficients above 0.90 in each of the three seasons. In F_2 hybrid material grown in 1934 the correlation coefficient between breaking strength and weight per unit length was 0.77, and in 1936 in similar material it was 0.90. Between means of F_3 progeny lines grown in 1936 the correlation coefficient was 0.82. Breaking-strength tests were not made in 1940 or later.

Frequency distributions of breaking strength for Kanred, Coppei, and the F_1 and F_2 populations of the hybrid between these varieties are given in table 7. The number of parent and F_1 plants tested was not adequate for accurate comparisons.
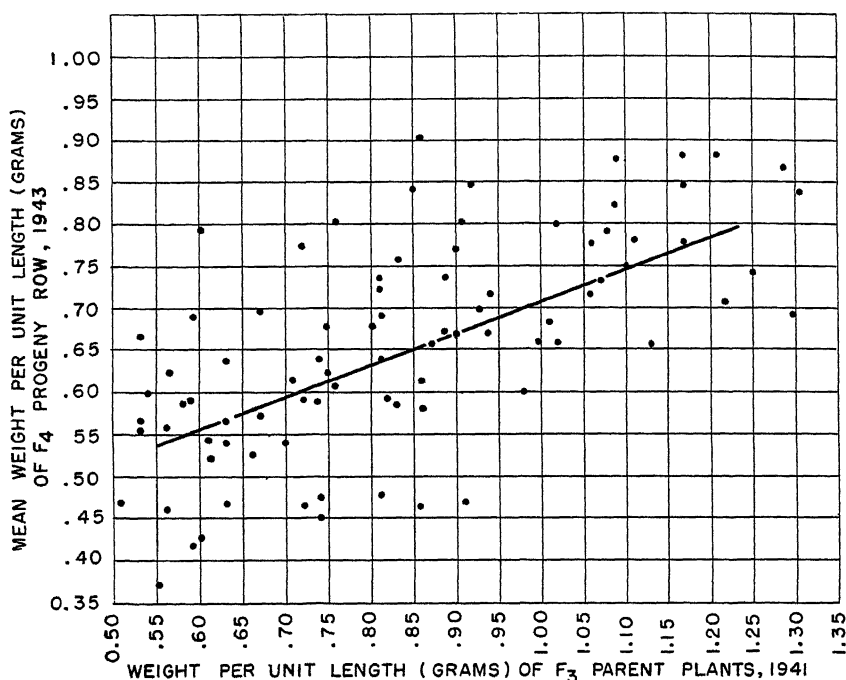
FIGURE 3.—Relation of weights per unit length of Kanred × Coppei F₃ wheat plants grown in 1941 and the means of their F₄ progeny lines grown in 1943, Denton, Tex.

The data presented in table 7 indicate that Kanred and Coppei differ significantly in mean breaking strength. Because the growing season was more favorable, the breaking strength of all material was higher in 1936 than in 1934. In fact, it probably was higher in 1936 than the records indicate for the Coppei parent and some of the hybrids because the machine was not properly adjusted for the higher values as shown by later tests. One result is an unusually large proportion of the population in the upper classes and an abnormally low standard deviation in 1936 as compared with other seasons and with the Kanred parent. This error was not discovered until the tests had been completed.

Breaking-strength tests also were made on the parents and on 153 F₃ progeny lines in 1936. Twenty plants were tested from each line, but only 25 spaced plants in single rows of Kanred and Coppei were tested. The study was continued on the F₄ generation in 1937, when 109 progeny lines were grown. Included in this group were the extremes in breaking strength of the F₃ lines. Others were selected at random. For comparison 9 progeny rows of Kanred and 5 of Coppei were grown. Frequency distributions for the means of F₃ progeny lines in 1936 and for the F₄ lines grown in 1937, together with those of the parental lines, are given in table 8. Since these progeny lines were not replicated, a generalized error could not be calculated and the breaking-strength and coefficient-of-variability classes in table 8 are merely arbitrary intervals.

TABLE 7.—*Frequency distribution for breaking strength of plants of parents and F_1 and F_2 populations of the wheat cross Kanred × Coppei, Denton, Tex., 1934 and 1936*

Year and parent or cross	Plants with indicated breaking strength (pounds) per 5 culms										Total plants	Mean	Standard deviation	Coefficient of variability
	3.51–4.00	4.01–4.50	4.51–5.00	5.01–5.50	5.51–6.00	6.01–6.50	6.51–7.00	7.01–7.50	7.51–8.00	8.01–8.50				
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Pounds	Pound	Percent
1934														
Kanred parent	3	5	7	3	3	0	0	0	0	0	21	4.85	0.785	16.19
Coppei parent	0	0	2	0	1	4	2	3	10	0	20	7.27	.771	10.61
Kanred × Coppei F₁	0	0	2	3	7	5	3	4	2	0	26	6.25	.832	13.31
Kanred × Coppei F₂	1	4	8	11	15	31	31	33	35	4	173	6.67	.988	14.82
1936														
Kanred parent	0	1	0	0	2	13	8	1	0	0	25	6.37	.553	8.68
Coppei parent	0	0	0	0	0	0	0	0	8	13	21	8.03	.109	1.35
Kanred × Coppei F₁	0	0	0	0	0	0	0	0	0	5	5	8.08	.029	.34
Kanred × Coppei F₂	0	0	0	8	12	18	22	27	64	34	185	7.23	.841	11.63

TABLE 8.—*Frequency distribution for breaking strength grouped by arbitrary coefficient-of-variability classes for means of parental and F_3 and F_4 progeny lines of the wheat cross Kanred $\times$ Coppei. Denton, Tex., 1936 and 1937*

Year and parent or cross	Coefficient-of-variability class	Lines with indicated breaking strength (pounds) per 5 culms								Total lines
		4 51–5 00	5.01–5.50	5.51–6 00	6.01–6 50	6.51–7 00	7 01–7.50	7.51–8.00	8 01–8.50	
1936	*Percent*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Kanred parent	7.1–10 0	0	0	0	1	0	0	0	0	1
Coppei parent	1.1–4 0	0	0	0	0	0	0	0	1	1
Kanred × Coppei F₃	1 1–4.0	0	0	0	0	0	0	3	1	4
	4.1–7 0	0	0	0	0	0	0	9	0	9
	7 1–10 0	0	0	0	0	1	16	14	0	31
	10.1–13 0	0	0	0	4	20	30	4	0	58
	13 1–16 0	0	0	0	8	20	7	0	0	35
	16.1–19.0	0	0	1	3	6	0	0	0	10
	19.1–22 0	0	1	2	3	0	0	0	0	6
Total		0	1	3	18	47	53	30	1	153
1937										
Kanred parent	7 1–10 0	1	1	0	0	0	0	0	0	2
	10.1–13 0	1	3	0	0	0	0	0	0	4
	13.1–16 0	2	0	1	0	0	0	0	0	3
Total		4	4	1	0	0	0	0	0	9
Coppei parent	7 1–10 0	0	0	0	0	0	3	0	0	3
	10 1–13.0	0	0	0	0	0	1	0	0	1
	13.1–16.0	0	0	0	0	1	0	0	0	1
Total		0	0	0	0	1	4	0	0	5
Kanred × Coppei F₄	7.1–10.0	0	0	4	5	7	1	0	0	17
	10.1–13.0	1	4	19	11	9	1	1	0	46
	13.1–16 0	2	4	17	14	3	0	0	0	40
	16.1–19.0	0	0	3	1	1	0	0	0	5
	19.1–22.0	0	0	0	0	0	1	0	0	1
Total		3	8	43	31	20	3	1	0	109

The F_3 progeny lines showed the same abnormal accumulation at the upper limit of the range of the breaking-strength machine as previously noted. Progeny lines having high breaking strength were in the lower classes of coefficient of variability and those having low breaking strength were in the upper classes of variability. The F_4 progeny lines ranged from the lower limits of the Kanred parent to one line having mean breaking strength greater than any Coppei parent line tested. The data indicate that in the F_4 lines there was no close relation between breaking-strength means and their coefficient-of-variability classes.

The correlation coefficient between breaking strength of F_2 parent plants grown in 1934 and means of their F_3 progeny lines grown in 1936 was 0.342 and that between F_3 parent plants of 1936 and means of their F_4 progeny lines grown in 1937 was 0.404. Although both figures are statistically significant, they are not sufficiently high to be of great value in selecting plants that would maintain high breaking strength in later generations. Smith (*11*) after a study of hybrid progenies in an oat cross came to a similar conclusion.

VARIABILITY AND INHERITANCE OF OTHER MORPHOLOGICAL CHARACTERS MEASURED

The characters total weight of culm, weight of head, diameter of culm, length of lower internode, and length of head were also measured.

Since previous studies of these characters in wheat varieties by Atkins (*1*, *2*) showed them to be less closely correlated with field lodging than are breaking strength or weight per unit length, the data are not reported in detail here. The means of the parents were significantly different statistically for each of these characters. Inheritance appeared to be controlled by multiple factors, resulting in the recovery of widely differing F_3 and F_4 progeny lines. Correlation coefficients between measurements of F_2 plants and the means of their F_3 progeny lines were 0.582 for diameter of culm, 0.694 for plant height, and 0.830 for head length, indicating that desired types of these characters can be selected with considerable success in the F_2 generation. Similar correlations for total weight of culm, weight of head, and length of lower internode were low. These results are in agreement with previous results obtained in studies of wheat varieties by Atkins (*1*) and in oats by Smith (*11*).

The inheritance of several spike characters, namely, density, length, awn development, and glume pubescence, also was studied with reference to their relation to weight per unit length. Since the inheritance of these spike characters has been studied by others, the detailed data for the present cross are not reported. The inheritance of awns was controlled by a single factor with incomplete dominance of the awnless condition in the F_1 generation. Pubescence of glumes was dominant and controlled by a single factor. One major factor controlled the inheritance of head type; but modifying factors were present, resulting in transgressive segregation for head length and the production of F_4 lines varying in head type and length from extremely short heads of the club type to heads of greater length than those of the Kanred parent.

INTERRELATION OF ALL CHARACTERS MEASURED

The interrelation of all characters measured was determined by means of correlation coefficients as shown in table 9. A study of the correlation coefficients in parental and hybrid populations indicates that many of these characters are associated to some extent. Weight per unit length was significantly correlated with total weight of culm and diameter of culm ($r=0.55$ to 0.83). In the Kanred parent the correlation coefficient between weight per unit length and plant height was 0.57, while in the Coppei parent it was only 0.02 and for all hybrid material it was low. In the Coppei parent weight per unit length was significantly correlated with length of lower internode ($r=0.62$), but in all other material similar correlations were nonsignificant.

Total weight of culm was most closely correlated with weight per unit length and with weight of heads. Diameter of culm was most closely correlated with weight per unit length, with weight of culm, and in the F_3 population with weight of heads. Plant height was significantly correlated in the F_2 and F_3 populations with length of head ($r=0.55$ to 0.64). Length of lower internode was independent of most other characters except in the F_3 population in which it was correlated with other characters to a moderate degree. Weight of head was most closely associated with weight per unit length, weight of culm, and diameter of culm. Length of head showed relatively low correlations with other characters except for plant height in the F_2 and F_3 populations.

TABLE 9.—*Correlation coefficients (r) for various plant characters of Kanred, Coppei, and Kanred × Coppei wheats, Denton, Tex., 1940 and 1941*

Year, parent or cross, and character correlated with character indicated in box	Basis	Weight of 5 culms (gm.)	Diameter of 5 culms (mm)	Plant height (cm.)	Length of lower internode (cm.)	Weight of 5 heads (gm.)	Head length (cm.)
1940							
Kanred parent							
Weight per unit length (gm)		0.67	0 60	0 57	0.07	0 42	0.03
Weight of 5 culms (gm)			.57	.C8	.05	.57	.45
Diameter of 5 culms (mm.)				.43	− 05	.32	.26
Plant height (cm.)	200 plants [1]				.02	46	.29
Length of lower internode (cm.)						.01	.08
Weight of 5 heads (gm.)							.40
Head length (cm.)							
Coppei parent:							
Weight per unit length (gm.)		64	.64	.02	.62	.59	.38
Weight of 5 culms (gm.)			.59	.42	03	.56	.17
Diameter of 5 culms (mm.)				.26	.00	.47	.26
Plant height (cm.)	do [1]				.16	.03	.16
Length of lower internode (cm)						.04	−.01
Weight of 5 heads (gm.)							.44
Head length (cm.)							
Kanred × Coppei F₂:							
Weight per unit length (gm.)		.55	.63	.21	.03	.46	.18
Weight of 5 culms (mm.)			62	35	.08	.48	21
Diameter of 5 culms (mm.)				.07	−.01	.42	.13
Plant height (cm.)	480 plants [2]				.21	.26	.55
Length of lower internode (cm.)						.01	.11
Weight of 5 heads (gm.)							.20
Head length (cm.)							
1941							
Kanred × Coppei F₃:							
Weight per unit length (gm.)		.78	.83	.34	.27	.64	.33
Weight of 5 culms (gm.)			.79	.58	.38	.69	.31
Diameter of 5 culms (mm.)				.37	.44	.68	.39
Plant height (cm)	87 progeny lines [3]				.49	.51	.64
Length of lower internode (cm.)						.40	.25
Weight of 5 heads (gm.)							.23
Head length (cm.)							

[1] 5-percent level of significance, 0 138; 1-percent level, 0.181.
[2] 5-percent level of significance, 0.088; 1-percent level, 0.115.
[3] 5-percent level of significance, 0.205; 1-percent level, 0.267.

CONTINGENCY STUDIES

The segregation of spike characters was recorded in the F_2 and F_3 populations. If any of these characters should be linked with genes determining lodging resistance as indicated by weight per unit length, then they might be useful as an aid in selecting plants in early generations. To determine the relation of these characters to weight per unit length, χ^2 tests for independence between weight per unit length and the spike characters pubescence of glumes, awn type, and head type and length were made for the 1940 F_2 data. In preparing contingency tables, no class division that had a lower expectancy than 6 was used. Results of the tests are given in table 10.

Pubescence of glumes, head type, and head length were independent of weight per unit length. Awn type was not entirely independent as a slightly larger proportion of awnless plants were in the higher weight-per-unit-length classes. However, the association was not high and awned types of relatively high weight per unit length were recovered. A similar relation was observed by Clark, Florell, and Hooker (4). In figures 4 and 5 are shown awned and awnless F_4 lines of the club-head and common types having relatively high and low mean weight per unit length. The parent varieties are shown for comparison. Coppei

TABLE 10.—x^2 *tests for independence of weight per unit length and spike characters in F_2 population of the wheat cross Kanred × Coppei, Denton, Tex., 1940*

Spike character	x^2	Degrees of freedom	P
Pubescence of glumes	7.254	4	0.05–0.10
Awn type	8.443	6	.20–.30
Head type	13.542	6	.02–.05
Head length	16.869	6	.01
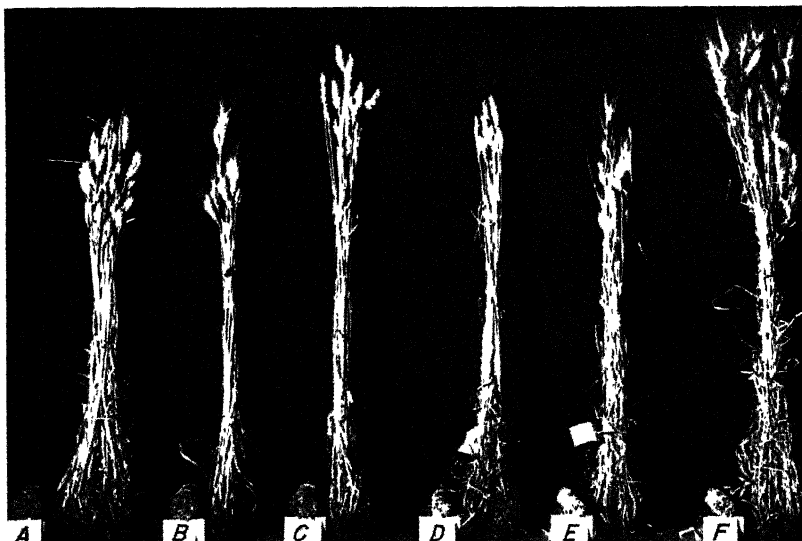
FIGURE 4.—Recovery of F_4 progeny lines of wheat having club heads and mean weight per unit length approaching that of each of the parental progeny lines, 1943: *A*, Coppei parent, 1.066 gm.; *B*, Kanred × Coppei (Sel. 73–350–18), 0.882 gm.; *C*, Kanred × Coppei (Sel. 73–183–10), 0.845 gm.; *D*, Kanred × Coppei (Sel. 73–141–17), 0.417 gm.; *E*, Kanred × Coppei (Sel. 73–390–31), 0.469 gm.; *F*, Kanred parent, 0.567 gm. The bundle of 100 culm sections at the base and left of each plant shows the relative size of culm of each progeny line.

progeny lines were winterkilled in 1943, but a few plants that survived in the rows are shown. Data for Coppei in 1941 were substituted for 1943, since none were available for 1943. As pointed out previously, no F_3 or F_4 progeny lines of extremely high weight per unit length were recovered, although some approached the Coppei lines. Progeny lines having mean weight per unit length within and below the range of the Kanred parent were recovered.

SUMMARY

Weight-per-unit-length measurements have been used successfully at Texas Substation No. 6, Denton, Tex., and several other agricultural experiment stations in the Midwest to evaluate varieties and strains of winter wheat for resistance to lodging. In the present study such tests were used on hybrid populations of a cross between the lodging-
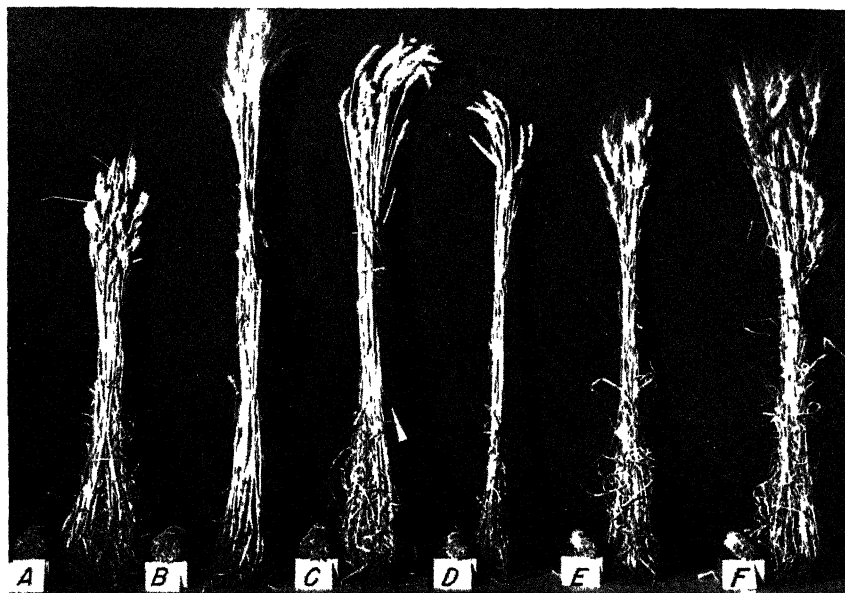
FIGURE 5.—Recovery of F_4 progeny lines of wheat having heads of the common type and mean weight per unit length approaching that of each of the parental progeny lines, 1943: *A*, Coppei parent, 1.066 gm.; *B*, Kanred × Coppei (Sel. 73–30–40), 0.883 gm.; *C*, Kanred × Coppei (Sel. 73–352–7), 0.887 gm.; *D*, Kanred × Coppei (Sel. 73–113–3), 0.464 gm.; *E*, Kanred × Coppei (Sel. 73–458–12), 0.370 gm.; *F*, Kanred parent, 0.567 gm. The bundle of 100 culm sections at the base and left of each plant shows the relative size of culm of each progeny line.

resistant club wheat variety, Coppei, and the lodging-susceptible common wheat variety, Kanred, to determine whether such tests can be made on individual plants in the early generations of a hybrid to select plants having high weight per unit length and the ability to transmit this characteristic to their progeny.

Tests conducted in several seasons on F_2, F_3, and F_4 populations and parental varieties of the cross indicate that environmental influences are large. This is shown by the range and variability of parents and F_1 plants, in which no genetic variability would be expected. The correlation coefficient between weight per unit length in F_2 parent plants and the means of their F_3 progeny lines was 0.609 in 1941. The similar correlation coefficient for the F_3 parent plants and their F_4 progeny lines in 1943 was 0.623. These coefficients indicate that the method may be used to select plants of high weight per unit length that will transmit this characteristic to their progeny. As no lodging occurred during the period of this experiment, the relation of weight per unit length in these hybrid lines to field lodging could not be determined.

Correlation coefficients of F_2 parent plants and their F_3 progeny lines were 0.582 for diameter of culm, 0.694 for plant height, and 0.830 for length of head. These correlation coefficients indicate, as with weight per unit length, that selection for these characters can be

made in early generations and that continued selection will lead to the desired type.   Correlation coefficients between F_2 parent plants and their F_3 progeny lines for weight of heads, length of lower internode, and weight of culms were low or nonsignificant.

The inheritance of awns was controlled by a single-factor pair, with incomplete dominance of the awnless character.   There was a low association between awnless head type and the higher weight-per-unit-length classes.   Pubescence of glumes was dominant in the F_1 and controlled by a single factor.   It was independent of weight per unit length.   One major-factor pair controlled the inheritance of head type; but transgressive segregation for head length, with the production of homozygous intermediate and extreme types, was observed in the F_3 and F_4 populations.   Therefore it is assumed that complementary factors were present.   Head length was independent of weight per unit length.

LITERATURE CITED

(1) ATKINS, I. M.
    1938. RELATION OF CERTAIN PLANT CHARACTERS TO STRENGTH OF STRAW AND LODGING IN WINTER WHEAT.   Jour. Agr. Res. 56: 99–120, illus.

(2) ――――
    1938. A SIMPLIFIED METHOD FOR TESTING THE LODGING RESISTANCE OF VARIETIES AND STRAINS OF WHEAT.   Amer. Soc. Agron. Jour. 30: 309–313, illus.

(3) BOSE, R. D., AZIZ, M. A., and BHATNAGAR, M. P.
    1937. STUDIES IN INDIAN BARLEYS. IV. THE INHERITANCE OF SOME ANATOMICAL CHARACTERS RESPONSIBLE FOR LODGING AND OF SOME EAR-HEAD CHARACTERS IN AN INTERSPECIFIC CROSS BETWEEN TWO PUSA BARLEYS.   Indian Jour. Agr. Sci. 7: 48–88, illus.

(4) CLARK, J. A., FLORELL, V. H., and HOOKER, J. R.
    1928. INHERITANCE OF AWNEDNESS, YIELD, AND QUALITY IN CROSSES BETWEEN BOBS, HARD FEDERATION, AND PROPO WHEATS AT DAVIS, CALIFORNIA.   U. S. Dept. Agr. Tech. Bul. 39, 40 pp., illus.

(5) GOULDEN, C. H., and NEATBY, K. W.
    1929. A STUDY OF DISEASE RESISTANCE AND OTHER VARIETAL CHARACTERS OF WHEAT—APPLICATION OF THE ANALYSIS OF VARIANCE, AND CORRELATION.   Sci. Agr. 9: 575–586.

(6) HAMILTON, D. G.
    1941. CERTAIN OAT CULM CHARACTERS AND THEIR RELATIONSHIP TO LODGING.   Sci. Agr. 21: 646–676, illus.

(7) KILDUFF, T.
    1933. INHERITANCE OF BUNT AND LOOSE SMUT REACTION AND OF CERTAIN OTHER CHARACTERS IN KOTA × RED BOBS AND GARNET CROSSES.   Canad. Jour. Res. 8: 147–172.

(8) PHILLIPS, M., DAVIDSON, J., and WEIHE, H. D.
    1931. STUDIES OF LIGNIN IN WHEAT STRAW WITH REFERENCE TO LODGING.   Jour. Agr. Res. 43: 619–626.

(9) RAMIAH, K., and DHARMALINGAM, S.
    1934. LODGING OF STRAW AND ITS INHERITANCE IN RICE (ORYZA SATIVA).   Indian Jour. Agr. Sci. 4: 880–894, illus.

(10) SALMON, S. C.
    1931. AN INSTRUMENT FOR DETERMINING THE BREAKING STRENGTH OF STRAW, AND A PRELIMINARY REPORT ON THE RELATION BETWEEN BREAKING STRENGTH AND LODGING.   Jour. Agr. Res. 43: 73–82, illus.

(11) SMITH, D. C.
    1934. CORRELATED INHERITANCE IN OATS OF REACTION TO DISEASES AND OTHER CHARACTERS.   Minn. Agr. Expt. Sta. Tech. Bul. 102, 38 pp., illus.

(12) TORRIE, J. H.
     1936. INHERITANCE STUDIES OF SEVERAL QUALITATIVE AND QUANTITATIVE
          CHARACTERS IN SPRING WHEAT CROSSES BETWEEN VARIETIES
          RELATIVELY SUSCEPTIBLE AND RESISTANT TO DROUGHT. Canad.
          Jour. Res. Sect. C, 14: 368–385, illus.
(13) WALDRON, L. R.
     1926. HYBRID SELECTIONS OF MARQUIS AND KOTA, A COMPARATIVE STUDY
          WITH REGARD TO DISEASE RESISTANCE, YIELD AND BAKING
          QUALITY. N. Dak. Agr. Expt. Sta. Bul. 200, 64 pp., illus.
(14) WELTON, F. A., and MORRIS, V. H.
     1931. LODGING IN OATS AND WHEAT. Ohio Agr. Expt. Sta. Bul. 471, 88
          pp., illus.

DIGESTIBLE NUTRIENTS AND METABOLIZABLE ENERGY IN RUSSIAN-THISTLE HAYS AND SILAGES[1]

By F. W. CHRISTENSEN, *animal nutritionist*, T. H. HOPPER, formerly *agricultural chemist*, and O. A. STEVENS, *associate botanist, North Dakota Agricultural Experiment Station* [2]

INTRODUCTION

For many years the Russian-thistle (*Salsola kali* L.) has been used as a feed for carrying livestock through the winter in the Great Plains area when little or no other forage was available. The dry years of 1934 and 1936 focused attention on this plant and emphasized the need of further information regarding its digestibility and usefulness as a feed. Since no determinations of digestibility were then available, this series of studies was undertaken. Later it was learned that digestion trials with cattle had been made by Cave, Riddell, and Hughes (*5*).[3]

THE RUSSIAN-THISTLE PLANT

DESCRIPTION

The compact, rounded form of the Russian-thistle plant is well known, but there is sometimes confusion in statements about the leaves becoming "spiny." The early leaves are as much as 2 or 3 inches long and are round in cross section. By the first of July, plants in the open may have reached a height of 1 to 1½ feet and are a mass of fleshy leaves, mostly with soft stems. About the middle of July the flowers begin to appear, and by the end of the month the plants are in full bloom. The plants continue to grow, the stems elongating and producing new branches and flowers until frost.

The leaves on the upper branches are not so long as those on the lower branches, and become shorter and shorter toward the ends of the branches. Flowers are borne singly at the nodes, each one just above the base of a leaf. Each flower is accompanied by two bracts, short, somewhat triangular, spine-tipped leaves about one-fourth of an inch long. On the upper branches the leaves themselves are reduced to the size of these bracts. On the lower stems the nodes may be as much as an inch apart, but toward the top of the plant they grow

Journal of Agricultural Research,   Vol. 76, Nos. 3 & 4
Washington, D. C.   Feb. 1 & 15, 1948
Key No. N. Dak.–22

773876—48——1   (73)

closer and closer together, so that the last few inches of each branch is crowded with "seeds," each one protected by the stem on one side and by the three spines on the other. The true leaves never have more than a very small spine, and a large proportion of them dry up or are eaten by insects.

The characteristic appearance of the upper branches with thick-set spines and winged fruits is not apparent until late August. In the lower, shaded part of the plant, the branches remain more leafy and succulent, so that large, old plants are by no means all hard and spiny.

The foregoing description is of plants growing in the open from seeds that germinate early in the spring. Those developing from seeds that germinate later in the season and from plants more or less shaded by other plants would be retarded in their development or would be variously modified. The chief difference probably would be in the size of the plant.

The Russian-thistle is especially well adapted to the dry plains region because of its ability to grow under dry conditions and to distribute its seeds when the mature plants are broken off and blown by the wind over the open country. Briggs and Shantz (*3*) found at Akron, Colo., that 336 pounds of water was used by the Russian-thistle in producing 1 pound of dry material. This is less than the quantity used by most other species, either weeds or crop plants, and much less than that used by wheat or flax. Dillman (*8*) obtained a still lower figure of 224 pounds at Mandan, N. Dak.

It is a matter of common observation that the Russian-thistle thrives during a dry season when most species make very poor growth. In 1934, when crop failure from drought was general in North Dakota, fields were covered with Russian-thistles, and they were common in the Red River Valley in the eastern part of the State where they are usually restricted to dry banks. In 1941, when moisture was more abundant, Russian thistles were remarkably inconspicuous. For a fuller account of this plant, see Stevens (*14*).

CHEMICAL COMPOSITION

As a part of this investigation, samples of Russian-thistle were collected from different parts of the State and at different stages of growth. Two series were secured from different soil types in the same general region at successive dates in 1936. The analyses of these samples are presented in table 1.

To facilitate comparison the data are calculated to a uniform water content of 15 percent. Wide variations in composition are noted. The ash varies from 12.07 percent to 24.36 percent and the protein $(N \times 6.25)$ from 7.93 percent to 20.84 percent. The crude fiber varies from 11.16 percent in young succulent plants to 26.21 percent in a sample collected at Fargo, September 3, 1936. The nitrogen-free extract varies from 26.95 to 39.31 percent, and the ether extract varies from 1.18 to 2.00 percent.

It is interesting to note, particularly in the Fargo and Leonard samples, that the ash is highest in the young, tender plants and lowest in the more mature plants. This is also noticeable to some extent in the analyses of other samples. Similarly, protein tends to be higher in the young plants and lower in the older ones. However, other factors such as season, rainfall, and soil, undoubtedly have an impor-

TABLE 1.—*Chemical composition of Russian-thistles collected in North Dakota in 1934, 1935, and 1936*

Source of sample	Date collected	Description of sample or site from which collected	Water	Ash	Crude protein	Crude fiber	N-free extract	Ether extract	Phosphorus
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent* [1]
Mapleton	July 23, 1934	2 feet high	15.00	15.74	8.80	23.54	35.65	1.27	0.136
Bedford	---do	18 inches high	15.00	13.32	9.78	24.57	36.14	1.18	.107
Page	---do	8-10 inches high	15.00	16.04	15.35	14.98	37.19	1.44	.149
Barrie	Aug. 3, 1934	12-18 inches high	15.00	12.07	7.93	24.45	39.31	1.24	.218
Mott	July 11, 1935	Succulent	15.00	24.36	18.11	11.16	29.38	1.99	
Do	---do	6 inches; crowded	15.00	18.90	17.48	15.38	32.06	1.16	
New England	Aug. 5, 1935	Field; rank	15.00	16.30	11.17	19.33	36.58	1.62	
Do	---do	Field; small	15.00	20.37	16.12	14.98	32.25	1.28	
Fargo	Sept. 13, 1935	Compost heap	15.00	15.61	14.12	22.98	30.78	1.51	
Do	---do	Railroad	15.00	12.41	13.85	24.95	32.20	1.50	
Do [1]	June 16, 1936	6-8 inches high	15.00	24.30	17.46	11.53	30.07	1.64	
Do [1]	July 1, 1936	10-12 inches, first flowers	15.00	22.09	20.15	12.74	28.02	2.00	
Do [1]	July 16, 1936	12-18 inches high	15.00	18.90	19.44	14.72	30.23	1.71	
Do [1]	Aug. 3, 1936		15.00	18.40	17.86	15.16	31.97	1.61	
Do [1]	Aug. 11, 1936		15.00	18.89	17.77	16.95	29.80	1.59	
Do [1]	Sept. 3, 1936		15.00	14.07	13.54	26.21	29.38	1.80	
Leonard [2]	July 3, 1936		15.00	18.41	20.84	17.06	26.95	1.75	
Do [2]	July 16, 1936		15.00	18.90	19.44	14.72	30.23	1.71	
Do [2]	Aug. 5, 1936		15.00	13.99	13.71	21.68	34.31	1.31	
Do [2]	Sept. 3, 1936		15.00	14.54	14.72	22.84	31.27	1.63	

[1] This series from the same location along a dirt road.
[2] From very sandy soil, but not identical locations.

tant effect on the protein content. For instance, one of the four samples collected in 1934 compared favorably in protein with those collected in 1935 and 1936, but the other three had decidedly less. Crude fiber was lowest in the young, tender plants and highest in the more mature ones. No definite relationship between the ether extract or nitrogen-free extract and the maturity of the plant was apparent.

In the samples of 1934 the sample from Barrie contained approximately twice as much phosphorus as the sample from Bedford.

Analyses of stems and leaves were made on one sample cut July 1, 1936, and of stems, leaves, and flowering tips on a sample cut July 15, 1936. From the data obtained (table 2) it is clear that the stems

TABLE 2.—*Chemical composition of stems, leaves, and floral tips of Russian thistles collected in North Dakota, 1936*

Date collected	Description of sample	Water	Ash	Crude protein	Crude fiber	N-free extract	Ether extract
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
July 1, 1936	Stems	15.00	17.75	12.44	19.87	33.26	1.68
Do	Leaves	15.00	20.51	21.78	7.68	32.45	2.58
July 15, 1936	Stems	15.00	11.97	7.51	29.12	35.23	1.17
Do	Leaves	15.00	23.09	13.60	8.92	36.69	2.70
Do	Floral tips	15.00	16.70	23.15	9.34	34.07	1.74

were decidedly lower in ash, protein, and ether extract than the leaves and much higher in crude fiber. The nitrogen-free extract was nearly the same in the stems and leaves. The flowering tips contained 23.15 percent protein, which is higher than that in either stems or leaves. This table shows considerable variation in the composition of different parts of the plant and helps to explain

differences in lots of hay cut at different stages of maturity and in different places.

EXPERIMENTAL PROCEDURE

ANIMALS

Wether lambs from the experiment station flock were used in these trials. A new group of 12 wethers was used each year. The individual wethers are identified by the letters, A, B, C, etc., followed by a numeral, thus: A–36, A–37, etc.

COLLECTION OF EXCRETA

A set of 12 portable metabolism stalls, or cages, with heavy screen floors and drain pans for collecting the urine were built for these trials. The cages were large enough to permit the animals considerable freedom in getting up and down, but not large enough to permit them to turn about. The feces were collected in bags made from ordinary duck, lined with hospital rubber sheeting. The bags were held in place with a light harness.

WEIGHING AND SAMPLING OF FEEDS AND EXCRETA

Each portion of the ration was weighed to the nearest gram morning and night. At each weighing a definite portion was set aside to make the composite sample that would be used for chemical analysis at the end of the 10-day metabolism trial. In those trials in which silages were fed definite amounts of the silage were weighed and dried daily and composited at the end of the trial for the chemical sample. The 24-hour collections of feces and urine were accurately weighed. The total feces were air-dried in a bulk-sample drier (*9*) and sampled for analysis after grinding. When dried in the bulk-sample drier there was no appreciable loss of nitrogen. Aliquots of the fresh urine samples were composited daily for analysis at the end of the trial. The composite urine samples were preserved by the addition of toluene and storage in a refrigerator.

METHODS OF CHEMICAL ANALYSIS

The chemical analyses of feeds, feed residues, and feces were made by the official methods of the Association of Official Agricultural Chemists (*2*). For the determination of the mineral constituents in the urine, the samples were wet-ashed with nitric acid. Before taking the digestions to dryness the excess nitric acid was removed by addition of hydrochloric acid. The micro method was used for the determination of calcium. Before precipitation of the magnesium as magnesium ammonium phosphate, the calcium was removed as sulfate in alcohol. The term "protein" as used in this publication means the nitrogen times the factor 6.25.

The energy values of the feeds and excreta were determined with a Parr adiabatic oxygen-bomb calorimeter. Before combustion the urine was dried over sulfuric acid in vacuum desiccators and the caloric values were corrected for nitrogen lost on drying. As the urine had low caloric values, it was necessary to add pellets of known weight of benzoic acid of known caloric value in order to obtain complete combustion in the oxygen bomb. These pellets were added just prior to combustion of the sample.

SCHEDULE OF METABOLISM TRIALS

The metabolism trials included determinations of digestibility of rations; nitrogen, calcium, phosphorus, and magnesium balances; and energy determinations on feeds and visible excreta. The trials were 10 days in length, preceded by preliminary periods of 10 days or more. In spite of care in adjusting the rations to the appetites of the test animals before the trials were begun, there were appreciable feed residues in some instances.

To facilitate the general account of the trials and the identification of particular trials, a schedule giving the trial numbers, year of experiment, identification numbers of the test animals, and dates of preliminary feeding periods and metabolism trials are given in table 3.

TABLE 3.—*Schedule of metabolism trials*

Trial No.	Year of experiment	Sheep No.	Feeds	Transitional and preliminary periods [1]	Metabolism periods [1].
23	1936	A–36, B–36, C–36, D–36, E–36, F–36.	Russian-thistle hay, coarse..	Feb. 4–16.....	Feb. 17–26.
24	1936	G–36, H–36, I–36, J–36, K–36.	Russian-thistle hay, fine....	.....do.........	Do.
39	1937	G–37, H–37, I–37.........	Russian-thistles, ground, and cane molasses.[2]	Mar. 13–22....	Mar. 23–Apr. 1.
33	1937	I–37, J–37, K–37.........	Russian-thistle-alfalfa silage.	Jan. 13–25......	Jan. 26–Feb. 4.
37	1937	A–37, B–37, C–37.........	Russian-thistle-molasses silage.	Mar. 13–22....	Mar. 23–Apr. 1.
38	1937	D–37, E–37, F–37.........	Russian-thistle-phosphoric acid silage.	.....do.........	Do.

[1] Dates are inclusive. [2] Ground Russian-thistle hay, 5 parts; cane molasses, 1 part; water, 3 parts.

FEEDS USED IN TRIALS

A detailed description of the feeds used in these trials is given because Russian-thistles are not grown as a regular crop, but volunteer generously under droughty conditions. They are generally obtained from fields where seeded grain crops have failed to germinate or grow because of drought; along fences or restricted areas in fields. For this reason Russian-thistle hays are usually not "pure."

Coarse Russian-thistles.—These thistles were cut from the fence row of a cornfield, September 21 to 23, 1935. The growth was rank, and the stems were coarse, but the plants were still green at the time of cutting. A mechanical separation of the air-dried thistles indicated 83.5 percent of Russian-thistles, 9.0 percent of straw and grass, 0.6 percent of cornstalks, and 6.9 percent of miscellaneous weeds.

Fine Russian-thistles.—These thistles were cut September 27, 1935, on a plot from which a crop of crested wheat hay had been cut about the middle of July. They were much finer stemmed than the coarse thistles and were not nearly so tall at the time of cutting. This lot was also a purer fodder than the coarse thistles. A mechanical separation showed 93.5 percent of thistles and 6.5 percent of straw or crested wheat stems.

Russian-thistle hay, ground.—This hay was cut near Wheelock in Williams County, N. Dak., August 1, 1936, from a field that had been seeded to wheat, but in which the wheat had largely blown out or had failed to germinate. The thistles were cut and stacked rather loosely the same day in stacks about 10 to 12 feet wide. They were baled August 19 to 23 from the stack and shipped to the experiment station. The hay was very dry at the time of baling. The

mechanical separation showed about 90 percent of thistles and 10 percent of wheat straw containing a few heads of wheat. These thistles were a little coarser than the "fine" thistles grown at the experiment station, but they may be considered a fair sample of Russian-thistle hay.

Russian-thistle-alfalfa silage.—The thistles for this silage were from a field containing a very thin and scattered stand of alfalfa. The proportion of alfalfa to thistles varied in different parts of the field, and, therefore, the amount of alfalfa could not be estimated accurately. From observation of the different loads it was estimated that from 15 to 20 percent of the silage material was alfalfa, with a sprinkling of rough pigweed and prickly lettuce. Ground barley was added at the rate of about 85 pounds to the ton of wilted thistles. Water was also added. A small experimental cement stave silo, 8 feet in diameter and 25 feet high, was filled between August 15 and August 18, 1934. The thistles were soft and pliable, only a small proportion having hard, sharp spines. The silo was not opened until January 13, 1937. Much of the silage at the top and around the sides of the silo was spoiled, but the center contained some good, well-preserved silage, which was fed in trial 33. Another attempt at making silage from the thistles likewise resulted in much spoilage, and this also occurred in one case where molasses was added to the silage material. Evidently, from these trials and the experience of farmers, it is rather difficult to make good silage from Russian-thistles.

Russian-thistle-molasses silage.—On October 24, 1936, some silage was made from dry, chopped thistles by moistening them with water at the rate of 2 pounds of water to 1 pound of dry thistles and molasses at the rate of 70 pounds per ton of wet material. After mixing, the wet material was packed into galvanized iron cans 2 feet in diameter and 5 feet deep by tamping with a heavy iron. The cans were then sealed with waterproof paper and a layer of dirt on top and stored in a fairly warm basement until March 13, 1937, when the silage was used for the digestion trials. After removing about 1 foot of moldy silage at the top, a clean, dark-brown silage of good odor was reached. This silage was fed in trial 37.

Russian-thistle-phosphoric acid silage.—This silage was made on February 13, 1937, by moistening some of the dry, chopped Russian-thistles of the 1935 crop with water at the rate of 2 pounds per pound of the dry material and adding syrup of phosphoric acid at the rate of 10 pounds per ton of wet material. The same kind of cans were used as for the thistle-molasses silage. The cans were opened on March 13, 1937, and after about 1 foot of dirt and moldy silage had been removed from the top, good, well-preserved silage was reached. This was dark brown in color and appeared a little drier than normal silage. After opening, the silage tended to heat and mold more quickly than the silage made with molasses. This suggests that a larger amount of phosphoric acid should have been added. This silage was fed in trial 38.

CHEMICAL COMPOSITION AND GROSS ENERGY OF FEEDS

The chemical composition and gross energy of the feeds is given in table 4. To save space the analyses of feed residues and excreta are omitted.

TABLE 4.—*Chemical composition of Russian-thistle hays and silages*

Feeds	Trial No.	Year of experiment	Dry matter	Ash	Crude protein	Crude fiber	N-free extract	Ether extract	Nitrogen	Calcium	Phosphorus	Magnesium	Energy per 100 pounds
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Therms*
Composition of dry matter													
Russian-thistle hay, coarse	23	1936	84.46	13.24	12.83	31.08	36.70	3.15	2.052	1.317	0.212	1.198	183.18
Russian-thistle hay, fine	24	1936	86.02	13.08	15.01	28.93	42.01	2.97	2.400	1.450	.204	1.873	180.93
Russian-thistle hay and molasses[1]	39	1937	55.36	15.36	12.62	20.35	50.06	1.61	2.020	2.758	.152	.901	170.67
Russian-thistle-alfalfa silage	33	1937	32.27	21.53	14.06	25.14	37.00	2.27	2.250	2.210	.174	1.110	175.51
Russian-thistle-molasses silage	37	1937	34.50	14.51	15.09	27.87	39.05	3.48	2.414	2.923	.209	1.586	181.59
Russian-thistle-phosphoric acid silage	38	1937	33.17	12.85	14.91	31.42	37.78	3.01	2.386	3.294	.573	1.852	184.37
Russian-thistle hay[2]	39	1937	87.24	16.72	14.39	23.30	44.19	1.41	2.303	[3]1.875	[3].188	[3].915	
Molasses, cane	39	1937	73.98	10.59	3.41		86.00		.546	1.939	.054	.431	162.12
Composition of feeds as fed													
Russian-thistle hay, coarse	23	1936	84.46	11.18	10.84	28.78	31.00	2.66	1.733	1.112	0.179	1.012	154.71
Russian-thistle hay, fine	24	1936	86.62	9.60	13.00	25.06	36.49	2.57	2.079	1.264	.177	1.622	156.72
Russian-thistle hay and molasses[1]	39	1937	55.36	8.50	6.99	11.27	27.71	.80	1.118	1.527	.081	.500	94.48
Russian-thistle-alfalfa silage	33	1937	32.27	6.95	4.54	8.11	11.94	.73	.726	.713	.056	.388	56.64
Russian-thistle-molasses silage	37	1937	34.50	5.01	5.21	9.62	13.47	1.20	.833	1.008	.072	.517	62.65
Russian-thistle-phosphoric acid silage	38	1937	33.17	4.26	4.95	10.42	12.53	1.01	.791	1.093	.190	.614	61.16
Russian-thistle hay[2]	39	1937	87.24	14.59	12.55	20.33	38.55	1.23	2.009	[3]1.036	[3].164	[3].798	
Molasses, cane	39	1937	73.98	7.83	2.52		63.62		.404	1.434	.040	.319	119.91

[1] As fed; ground Russian-thistles, 5 parts; cane molasses, 1 part; water, 3 parts.
[2] Through a misunderstanding, no separate sample of the thistles was taken in trial 39. These analyses are the average of 2 other samples from the same lot of hay.
[3] The mineral analysis is from a single sample on the same lot of hay.

RATIONS AND WATER CONSUMED

Table 5 shows the amount of feed offered to each sheep during the trial, the amount of the feed residues, and the water drunk. This is given in place of a more detailed table showing how much of each nutrient was consumed, excreted, and digested. The more detailed table is omitted to save space.

TABLE 5.—*Average rations offered, feed residues, and water drunk during metabolism trials*

Trial No.	Year of experiment	Ration, residues, and water	Sheep No.					
			A-36	B-36	C-36	D-36	E-36	F-36
23	1936	Russian-thistles, coarse_____grams__	250.0	280.0	200 0	250 0	320.0	320.0
		Uneaten feed residues_____do____	12.4	11 2	70.0	8 9	4.3	3 6
		Water drunk_____do____	485.0	417.0	503.0	608.0	803.0	748.0
			Sheep No.					
			G-36	H-36	I-36	J-36	K-36	L-36
24	1936	Russian-thistles, fine_____do____	280.0	200.0	250 0	200.0	200.0	None
		Uneaten feed residues_____do____	.7	1.2	24.9	1.9	18.6	None
		Water drunk_____do____	662.0	526.0	608.0	594.0	626.0	962.0
			Sheep No.					
			G-37	H-37	I-37			
39	1937	Russian-thistles, ground, 5 parts; molasses, 1 part; water, 3 parts_____do____	1,200.0	1,100.0	700.0			
		Uneaten feed residues_____do____	7 8	.6	.7			
		Water drunk_____do____	2,127.0	2,046.0	1,610.0			
			Sheep No.					
			I-37	J-37	K-37			
33	1937	Russian-thistle-alfalfa silage_____do____	1,100.0	1,300.0	1,400.0			
		Uneaten feed residues_____do____	99.4	1 5	1.0			
		Water drunk_____do____	748.0	2,046.0	2,799.0			
			Sheep No.					
			A-37	B-37	C-37			
37	1937	Russian-thistle-molasses silage__do____	700.0	1,200.0	800.0			
		Uneaten feed residues_____do____	5.6	.1	.5			
		Water drunk_____do____	726.0	494.0	1,275.0			
			Sheep No.					
			D-37	E-37	F-37			
38	1937	Russian-thistle-phosphoric acid silage_____do____	700.0	800.0	600.0			
		Uneaten feed residues_____do____	3.9	46.5	3.7			
		Water drunk_____do__ _	608.0	1,170.0	572.0			

It was found that the sheep would not eat the dry, chopped thistles, and, therefore, in trials 23 and 24, the morning and evening portions were weighed into 25-pound lard cans, sprinkled with distilled water, and allowed to soak until the next day. Even when thus soaked,

the sheep ate only from 200 to 320 gm. of the dry thistles per head daily, which was not enough to maintain their weight.

In trial 39 the ground thistles were moistened with cane molasses and water in the proportions of 5 parts of thistles, 1 part of molasses, and 3 parts of water. With this treatment, the sheep ate from 389 to 667 gm. of thistles and from 78 to 133 gm. of molasses per head daily. Sheep I–37, which ate the smallest amounts, lost a little weight during the trial, but the other two sheep gained a little. In trials 33, 37, and 38 all the sheep lost weight, indicating that the rations were unpalatable and that not enough was eaten for maintenance.

The water drunk by the sheep was from the Fargo water system. Daily samples were taken to form a composite sample for analysis. The analyses for calcium and magnesium are given in table 6. The analyses failed to show any phosphorus in the water.

TABLE 6.—*Calcium and magnesium in drinking water*

Feeds	Trial No.	Year of experiment	Minerals in water [1]	
			Calcium	Magnesium
			P. p. m.	*P. p. m.*
Russian-thistle hay, coarse	23	1936	18. 7	65. 1
Russian-thistle hay, fine	24	1936	18. 7	65. 1
Russian-thistle hay, ground, and molasses	39	1937	10. 7	58. 7
Russian-thistle-alfalfa silage	33	1937	17 1	45. 2
Russian-thistle-molasses silage	37	1937	10. 7	58. 7
Russian-thistle-phosphoric acid silage	38	1937	10. 7	58. 7

[1] No phosphorus was present in the water.

RESULTS OF METABOLISM TESTS
APPARENT DIGESTIBILITY OF FEEDS

The percentage apparent digestibility of the feeds, as determined by the individual sheep in the different trials, is given in table 7. The average coefficients are summarized in table 8.

TABLE 7.—*Percentage apparent digestibility of feeds*

Trial No.	Sheep No.	Feeds	Dry matter	Organic matter	Crude protein	Crude fiber	N-free extract	Ether extract
			Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
23	A–36	Russian-thistle hay, coarse	51. 2	52. 1	70. 0	40. 6	53. 8	73. 9
	B–36		46. 9	47. 3	67. 5	33. 0	50. 5	76. 4
	C–36		54. 1	53. 4	67. 6	41. 8	55. 2	79. 0
	D–36		45. 2	44. 9	64. 4	31. 2	47. 7	75. 8
	E–36		47. 6	48. 8	63. 9	36. 6	52. 9	70. 2
	F–36		44. 7	44. 2	66. 2	31. 7	45. 6	73. 8
24	G–36	Russian-thistle hay, fine	44. 5	47. 7	66. 4	32. 0	50. 5	65. 0
	H–36		46. 6	49. 4	68. 0	34. 3	51. 7	68. 6
	I–36		48. 6	50. 8	67. 1	35. 2	54. 3	70. 7
	J–36		46. 7	49. 6	68. 6	37. 5	50. 0	64. 7
	K–36		48. 4	52. 3	66. 5	38. 4	54. 9	70. 8
39	G–37	Russian-thistle hay, ground, and molasses.[1]	61. 8	61. 9	64. 4	44. 8	68. 0	64. 2
	H–37		61. 3	61. 3	64. 5	41. 0	68. 5	71. 4
	I–37		62. 9	63. 1	65. 0	44. 9	70. 0	66. 1
33	I–37	Russian-thistle-alfalfa silage [2]	50. 0	48. 1	36. 8	48. 6	51. 8	50. 0
	J–37		49. 5	46. 9	35. 0	46. 7	51. 4	49. 5
	K–37		50. 8	48 0	39. 9	46. 5	52. 1	48. 5
37	A–37	Russian-thistle-molasses silage [3]	43. 3	42. 9	59. 6	31. 4	41. 7	75. 6
	B–37		41. 6	42. 4	60. 3	30. 5	41. 6	70. 1
	C–37		44. 1	45. 8	62. 9	32. 2	45. 8	80. 2
38	D–37	Russian-thistle-phosphoric acid silage [4]	39. 2	41. 6	58. 1	31. 7	40. 1	81. 4
	E–37		35. 0	37. 0	50. 4	29. 1	34. 6	80. 0
	F–37		38. 7	41. 0	58. 2	33. 1	34. 5	80. 0

[1] Ground Russian-thistle hay, 5 parts; cane molasses, 1 part; water 3 parts.
[2] The fresh silage material contained some alfalfa, estimated at about 15 percent by weight.
[3] Molasses was added at the rate of about 70 pounds per ton of wet material.
[4] Phosphoric acid was added at the rate of 10 pounds per ton of wet material.

TABLE 8.—*Coefficients of apparent digestibility of Russian-thistle hays and silages*

Feeds	Trial No.	Indi-vidual trials	Year of experi-ment	Dry matter	Organic matter	Crude protein	Crude fiber	N-free extract	Ether extract
		Number		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Russian-thistle hay, coarse____	23	6	1936	47.6	47 8	66.4	35.1	50.4	74.6
Russian-thistle hay, fine_____	24	5	1936	46 8	49.8	67.3	35.2	52.2	67.9
Russian-thistle hay ground and molasses [1]_____	39	3	1937	61 9	62.0	64.6	43.4	68.7	67.3
Russian-thistle-alfalfa silage___	33	3	1937	50.2	47.6	37.4	47.0	51.8	49.2
Russian-thistle-molasses silage_____	37	3	1937	42.8	43.5	60.9	31.2	42.9	74.5
Russian-thistle-phosphoric acid silage_____	38	3	1937	37.6	39.8	55.4	31.2	37.4	80.5

[1] Ground Russian-thistle hay, 5 parts; cane molasses, 1 part; water 3 parts.

The average digestion coefficients for the coarse and fine Russian-thistles do not differ greatly. Except for a slightly lower coefficient for dry matter (difference −0.8), and for ether extract (−6.7), all the coefficients are slightly higher for the fine thistles, with differences as follows: Organic matter, +2.0; protein, +0.9; fiber, +0.1; and nitrogen-free extract, +1.8.

It is not possible to observe directly the effect of molasses upon the digestibility of the thistles, since the thistles fed in trial 39 were from a different crop. However, the coefficients for dry matter, organic matter, crude fiber, and nitrogen-free extract are higher, and the protein and ether extract are somewhat lower than they are for the thistles without the molasses.

Compared to the Russian-thistle hay, the Russian-thistle-alfalfa silage shows higher digestibility for dry matter and crude fiber, with approximately the same digestibility of organic matter and nitrogen-free extract, but decidedly lower digestibility of protein and ether extract. Apparently, ensiling and storing the material for about 2½ years had the effect of greatly reducing the digestibility of the protein. (See table 7.)

The Russian-thistle-molasses silage shows somewhat lower coefficients of digestibility than the hay in trial 24 for all nutrients except ether extract. The effect of the molasses and ensiling was to lower the digestibility of the fine thistles.

The Russian-thistle-phosphoric-acid silage, made from the same lot of thistles as the thistle-molasses silage, gave lower coefficients for dry matter, organic matter, crude protein, and nitrogen-free extract, but identical coefficients for crude fiber and higher digestibility for ether extract. It might be inferred from this that the phosphoric acid had, in general, a depressing effect on digestibility. In other tests at this station on leafy spurge there was no such effect, and, therefore, such a conclusion is not justified at this time.

<div align="center">NITROGEN BALANCES</div>

Table 9 gives the nitrogen balances of the individual sheep during the trials. These balances were obtained in order to determine the condition of the sheep while on experiment and to furnish data for the calculation of metabolizable energy. Only two sheep had positive nitrogen balances.

TABLE 9.—*Daily nitrogen balance of the experimental sheep*

Trial No.	Sheep No.	Feeds	Nitrogen in—			Nitrogen balance
			Feeds	Feces	Urine	
			Grams	*Grams*	*Grams*	*Grams*
	A-36		4.20	1.27	5.68	−2.75
	B-36		4.73	1.53	5.44	−2.24
23____	C-36	Russian-thistle hay, coarse_____	2 32	.75	4.60	−3.03
	D-36		4.22	1.50	5.09	−2.37
	E-36		5.49	1.99	6.42	−2.92
	F-36		5.49	1.85	6.34	−2.70
	G-3b		5.81	1.95	6.12	−2.26
	H-36		4.14	1.33	5.48	−2.67
24____	I-36	Russian-thistle hay, fine_____	4.63	1.51	5.63	−2.51
	J-36		4.13	1.30	5.71	−2.88
	K-36		3.82	1.28	5.29	−2.75
	G-37		13.32	4.74	6.99	+1.59
39____	H-37	Russian-thistle hay, ground, and molasses_____	12.29	4 37	6.85	+1.07
	I-37		7.82	2 74	5.21	−.13
	I-37		6.14	3.88	4.71	−2.45
33____	J-37	Russian-thistle-alfalfa silage_____	9.41	6.11	7.41	−4.11
	K-37		10.15	6.10	7.77	−3.72
	A-37		5.71	2.30	4.06	−.65
37____	B-37	Russian-thistle-molasses silage_____	9 99	3.96	6.37	−.34
	C-37		6 65	2.46	4.58	−.39
	D-37		5.46	2.29	4.33	−1.16
38____	E-37	Russian-thistle-phosphoric acid silage_____	5.41	2.68	4.37	−1.64
	F-37		4.70	1.96	4.55	−1.81

METABOLIZABLE ENERGY IN FEEDS AND RATIONS

The gross energy in the feed, feces, and urine was determined directly by means of a Parr adiabatic oxygen-bomb calorimeter. The energy in methane was computed by the method of Armsby (*1*) according to which 100 gm. of digestible carbohydrates produce 4.5 gm. of methane having an energy value of 60.1 large calories. The energy in the urine was corrected for gain or loss of protein by the use of Rubner's value for protein nitrogen as employed by Armsby (*1*). The energy data on all the rations are given in table 10.

From a study of the values obtained by various investigators, Armsby (*1*) found that the metabolizable energy per pound of digestible organic matter ranged from 1.50 to 1.70 therms per pound of digestible organic matter for roughages, with an average of 1.60 therms; for mixed rations, the values ranged from 1.58 to 1.87 therms, with an average of 1.66 therms. Christensen and Hopper (*7*), in trials with cattle, found a range of 1.67 to 1.81 therms per pound of digestible organic matter in 25 trials on roughages and a range of 1.72 to 1.84 therms on mixed rations. The average was 1.737 therms for the roughages and 1.767 for the mixed rations. On two prairie hays Christensen and Hopper (*6*) found the metabolizable energy per pound of digestible organic matter to be 1.432 and 1.514 therms. Similar low values have also been found on certain other feeds (unpublished data).

As shown in table 10, the metabolizable energy per pound of digestible organic matter ranges from 1.465 to 1.796 therms, with an average of 1.614 therms. It is not clear why the coarse thistles gave an average of 1.630 therms of metabolizable energy per pound of digestible organic matter and the fine thistles a lower value of 1.528 therms. On a dry basis the coarse thistles contained 13.24 percent of ash and 183.18 therms of metabolizable energy per 100 pounds, as compared to 11.08

TABLE 10.—*Gross energy of rations, losses in excreta, and metabolizable energy*

Trial No.	Sheep No.	Feeds	Gross energy of feed	Losses— In feces	Losses— In urine	Losses— As methane	Metabolizable	Percentage losses of energy— In feces	Percentage losses of energy— In urine	Percentage losses of energy— As methane	Metabolizable energy	Metabolizable energy Per pound of digestible organic matter	Metabolizable energy Per pound of digestible nutrients
			Therms	*Therms*	*Therms*	*Therms*	*Therms*	*Percent*	*Percent*	*Percent*	*Percent*	*Therms*	*Therms*
23	A-36	Russian-thistle hay, coarse	0.803	0.400	0.042	0.040	0.321	49.81	5.23	4.98	39.98	1.621	1.514
	B-36		.910	.497	.041	.040	.332	54.62	4.51	4.39	36.48	1.626	1.516
	C-36		.415	.197	.028	.021	.169	47.47	6.75	5.06	40.72	1.623	1.496
	D-36		.818	.463	.042	.034	.279	56.60	5.13	4.16	34.11	1.599	1.484
	E-36		1.075	.597	.050	.051	.407	52.74	4.65	4.74	37.80	1.636	1.536
	F-36		1.077	.606	.050	.044	.377	56.27	4.64	4.09	35.00	1.672	1.551
		Average						52.02	5.16	4.67	37.36	1.630	1.516
24	G-36	Russian-thistle hay, fine	.964	.523	.049	.044	.348	54.25	5.08	4.56	36.11	1.540	1.456
	H-36		.686	.361	.088	.033	.254	52.62	5.54	4.81	37.03	1.540	1.392
	I-36		.774	.397	.047	.039	.291	51.29	6.07	5.04	37.60	1.499	1.415
	J-36		.683	.352	.088	.033	.260	51.54	5.50	4.83	38.07	1.562	1.477
	K-36		.621	.300	.039	.032	.241	49.76	6.28	5.16	38.81	1.508	1.418
		Average						51.89	5.71	4.88	37.52	1.528	1.432
36	G-37	Russian-thistle hay, ground, with molasses	2.471	1.007	.147	.170	1.147	40.75	5.95	6.88	46.42	1.512	1.476
	H-37		2.299	.970	.135	.156	1.028	42.38	5.90	6.82	44.91	1.476	1.436
	I-37		1.455	.591	.094	.103	.667	40.62	6.46	7.08	45.84	1.465	1.428
		Average						41.25	6.10	6.93	45.72	1.484	1.447
33	I-37	Russian-thistle-alfalfa silage	1.024	.543	.042	.049	.390	53.03	4.10	4.70	38.09	1.787	1.726
	J-37		1.618	.883	.070	.077	.588	54.57	4.33	4.76	36.34	1.733	1.670
	K-37		1.745	.923	.066	.084	.672	52.89	3.78	4.81	38.51	1.791	1.728
		Average						53.50	4.07	4.79	37.65	1.770	1.708
37	A-37	Russian-thistle-molasses silage	.947	.537	.044	.036	.330	56.71	4.65	3.80	34.85	1.728	1.587
	B-37		1.657	.929	.072	.062	.594	56.07	4.35	3.74	35.85	1.796	1.655
	C-37		1.103	.597	.049	.044	.413	54.13	4.44	3.99	37.44	1.736	1.595
		Average						55.64	4.48	3.84	36.05	1.753	1.612
38	D-37	Russian-thistle-phosphoric acid silage	.930	.564	.046	.035	.285	60.65	4.95	3.76	30.65	1.563	1.429
	E-37		.907	.559	.043	.030	.240	64.94	5.29	3.31	26.46	1.515	1.379
	F-37		.795	.470	.039	.029	.248	60.25	4.91	3.65	31.19	1.609	1.485
		Average						61.95	5.05	3.57	29.43	1.502	1.434

percent of ash and 180.93 therms of energy in the fine thistles. The ground Russian-thistles with molasses showed the lowest value of all,· 1.484 therms per pound of digestible organic matter. No explanation

TABLE 11.—*Dry matter, digestible crude protein, total digestible nutrients, and metabolizable energy in 100 pounds of feed as fed and in dry matter (on apparent digestibility basis)*

Feeds	Trial No.	In- divid- ual trials	Year of ex- peri- ment	As fed					In dry matter		
				Dry mat- ter	Di- gest- ible crude pro- tein	Total digest- ible nutri- ients	Nu- tri- trive ratio 1:	Metab- olizable energy	Di- gest- ible crude pro- tein	Total digest- ible nutri- ents	Metab- olizable energy
		Num- ber			*Per- cent*	*Per- cent*	*Per- cent*		*Therms*	*Per- cent*	*Per- cent*	*Therms*
Russian-thistle hay, coarse.	23	6	1936	1 84. 46	7. 2	37. 4	4. 2	57. 80	8. 5	44. 3	68. 44
Russian-thistle hay, fine...	24	5	1936	1 86. 62	8. 7	40. 5	4. 6	58. 80	10. 1	46. 7	67. 88
Russian-thistle hay, ground, with molasses...	39	3	1937	55. 36	4. 5	29. 8	6. 6	43. 20	8. 2	53. 8	78. 03
Russian-thistle-alfalfa silage..................	33	3	1937	32. 37	1. 7	12. 5	6. 3	21. 32	5. 3	38. 8	66. 08
Russian-thistle-molasses silage..................	37	3	1937	34. 50	3. 2	14. 0	3. 4	22. 58	9. 2	40. 5	65. 46
Russian-thistle-phosphoric acid silage..............	38	3	1937	33. 17	2. 8	12. 5	3. 5	18. 00	8. 3	37. 7	54. 26

[1] On air-dry basis; not including water used in moistening thistles.

for this is apparent, since the total digestible nutrients are high (table 11).

The metabolizable energy per pound of total digestible nutrients in these trials ranges from 1.379 to 1.728 therms, with an average of 1.516. This is a little lower than the average value of 1.616 therms reported by Kriss (*10*).

DIGESTIBLE NUTRIENTS IN FEEDS AND RATIONS

The results of the several trials to determine digestible nutrients and metabolizable energy in the feeds are summarized in table 11. For convenience in using the data and making comparisons, the digestible nutrients and metabolizable energy are given for the feeds as fed as well as on a dry-matter basis.

Morrison's (*13*) average of all analyses for red clover hay, computed to a dry basis, is 7.9 percent of digestible protein and 58.8 percent of total digestible nutrients. From this it is evident that the Russian-thistle hay contains more digestible protein than does red clover hay, but about 12 to 14 percent less of total digestible nutrients.

Christensen and Hopper (*7*), in seven individual trials on corn silage, found 5.3 percent of digestible protein and 69.7 percent of total digestible nutrients on a dry basis. Morrison's (*13*) average of all analyses, computed to dry basis, is 4.6 percent digestible protein and 66.1 percent of total digestible nutrients. The total digestible nutrients in the three Russian-thistle silages range from 37.7 to 40.5 percent, which is considerably lower than in the corn silage. The digestible protein in the thistle silages ranges from 5.3 to 9.2 percent, which is, in general, somewhat higher than that in corn silage.

The metabolizable energy in the two thistle hays and silages is similar except for the thistle-phosphoric-acid silage. which is con-

siderably lower on an air-dry basis. This low value is due to the relatively large loss of energy in the feces (table 10).

An important consideration in evaluating any feed is its palatability and bulkiness. If a feed is unpalatable, an animal may not eat enough for its needs, or, if it is too bulky, the animal may lack capacity for consuming enough to meet its requirements. In these trials the Russian-thistle hays and silages proved rather unpalatable. In only two instances did the sheep eat enough to show a gain in weight, and that was on the ground thistles with molasses. Data on the average live weights, average daily gains or losses, and the dry matter, digestible protein, total digestible nutrients, and metabolizable energy in the rations are presented in table 12.

Brody, Procter, and Ashworth (*4*) give the maintenance requirement of a 100-pound sheep as 59 gm. of digestible protein, 572 gm., or 1.261 pounds, of total digestible nutrients, and 2.282 therms of energy, presumably metabolizable, on the basis of 1 pound of total digestible nutrients as equivalent to 1,812 calories. Kriss (*10*) gives 1.616 therms of metabolizable energy per pound of total digestible nutrients as the average value for mixed rations of cows. If we multiply 1.261 pounds of total digestible nutrients by 1.616, the requirement for metabolizable energy becomes 2.038 therms.

On the basis of either of the foregoing requirements, none of the sheep received enough total digestible nutrients or metabolizable energy for maintenance; yet sheep G–37 and H–37 in trial 39 showed some gains during the trial. Armsby (*1*) reviewed the available data and estimated the maintenance requirement of metabolizable energy from respiration experiments as 1.322 therms and from live weight experiments as 1.368 therms. Mitchell, Kammlade, and Hamilton (*11*) found the maintenance requirement for metabolizable energy in one series of trials with sheep to be 1.864 therms on alfalfa, 1.521 on clover, and 1.507 on timothy hay. In another series they found the requirement to be 1.382 therms for alfalfa and 1.149 for timothy hay.

From 11 respiration trials at the New Hampshire Experiment Station, Ritzman [4] estimates the maintenance requirement of adult sheep at 1.686 therms per 100 pounds of live weight. If five experiments at low temperatures are omitted, the figure becomes 1.820 therms. These lower values probably come closer to the actual requirements for sheep confined in metabolism cages than the higher values, but, even so, the sheep on the Russian-thistle hays alone (trials 23 and 24) ate only about one-fourth to one-third as much as was necessary for maintenance.

Two of the three sheep on ground Russian-thistles moistened with diluted molasses (trial 39) ate enough to show some gains, and they were the only ones that received enough protein to show positive nitrogen balances (table 9).

The sheep on the Russian-thistle-alfalfa silage (trial 33) and those on the thistle-molasses silage (trial 37) in general consumed somewhat more of the total digestible nutrients and metabolizable energy than those on the Russian thistles alone, indicating that these silages were somewhat more palatable. Those on the Russian-thistle-phosphoric

TABLE 12.—*Live weights, digestible nutrients, and metabolizable energy in rations and per hundred pounds live weight*

Trial No.	Sheep No.	Feeds	Live weight	Average daily gain or loss	Feed consumed per day per head					Feed consumed per hundred pounds live weight (W 7/3)			
					Dry matter	Digestible protein	Total digestible nutrients	Nutritive ratio 1:	Metabolizable energy	Dry matter	Digestible protein	Total digestible nutrients	Metabolizable energy
			Pounds	*Pounds*	*Grams*	*Grams*	*Grams*		*Therms*	*Grams*	*Grams*	*Grams*	*Therms*
23	A-36	Russian-thistle hay, coarse	85.0	−0.50	199.5	18.4	96.2	4.2	0.321	224.6	20.7	108.3	0.361
	B-36		71.6	−.56	226.0	19.9	99.2	4.0	.332	288.5	25.4	120.6	.424
	C-36		67.9	−.59	103.6	9.8	51.3	4.2	.169	137	13.0	68.1	.224
	D-36		66.6	−.56	202.9	17.0	85.2	4.0	.279	273.1	23.9	114.7	.375
	E-36		82.7	.47	266.3	21.9	120.0	4.5	.407	305.9	25.2	137.8	.465
	F-36		76.2	.53	266.9	22.7	110.0	3.9	.377	325.4	22.7	134.1	.460
		Average								299.2	22.5	114.9	.385
24	G-36	Russian-thistle hay, fine	69.8	.59	241.8	24.1	108.5	3.5	.318	314.4	41.3	141.1	.465
	H-36		63.7	.47	172.1	17.6	79.0	3.5	.274	230.2	24.5	111.1	.353
	I-36		80.6	.56	193.9	19.4	93.0	3.8	.291	227.0	23.7	100.9	.341
	J-36		73.8	.59	171.4	17.7	79.7	3.5	.290	214.0	22.1	99.5	.325
	K-36		73.8	.63	156.2	15.9	76.9	3.8	.241	223.0	22.7	109.8	.344
		Average								213.5	24.7	114.1	.364
39	G-37	Russian-thistle hay, ground, with molasses	69.5	+.18	657.0	53.6	352.3	5.6	1.147	856.9	69.9	450.5	1.496
	H-37		71.5	+.28	608.4	49.5	324.7	5.6	1.028	777.4	63.1	414.9	1.314
	I-37		75.0	−.11	386.8	31.7	211.8	5.7	.667	477.1	30.1	261.3	.823
		Average								703.8	67.4	378.6	1.211
33	I-37	Russian-thistle-alfalfa silage	72.7	−.29	201.2	14.1	102.3	6.3	.390	329.7	17.8	129.1	.492
	J-37		86.9	.02	418.1	20.6	159.8	6.8	.588	463.3	28.8	177.1	.652
	K-37		82.5	−.19	450.9	25.3	176.3	6.0	.672	518.8	29.1	202.9	.773
		Average								437.3	23.2	163.7	.639
37	A-37	Russian-thistle-molasses silage	45.5	−.08	236.4	21.2	94.4	3.5	.330	420.1	37.7	107.7	.586
	B-37		56.5	−.18	414.0	37.7	162.8	3.3	.594	628.7	57.2	247.0	.901
	C-37		43.4	.10	275.5	26.1	117.4	3.5	.413	503.7	48.0	215.9	.760
		Average								518.3	47.6	210.2	.749
38	D-37	Russian-thistle-phosphoric acid silage	55.2	−.18	228.6	19.8	90.0	3.6	.285	352.8	30.6	138.9	.440
	E-37		61.0	.35	222.9	17.1	79.1	3.6	.240	319.7	21.5	113.5	.314
	F-37		57.2	.33	195.6	17.1	75.9	3.4	.218	291.0	25.7	114.1	.373
		Average								322.2	24.9	122.2	.386

acid silage (trial 38) consumed about the same amounts of total digestible nutrients and metabolizable energy as those on the hays alone, indicating that this silage was less palatable than the other two.

It might appear from the data of table 12 that the Russian-thistles are of little or no value as feed for sheep. However, in another phase of this project bred ewes fed chopped Russian-thistles moistened with water and molasses as the only, or principal, roughage, supplemented with barley, produced healthy, vigorous lambs (unpublished data). The metabolism trials show that the Russian-thistles have considerable nutritive value, which can be utilized in times of feed emergency.

CALCIUM, PHOSPHORUS, AND MAGNESIUM BALANCES

Although this project was not planned to determine the mineral requirements of sheep, it seemed desirable to obtain information on the utilization of calcium, phosphorus, and magnesium along with the other data. Table 13 gives the data on the intake, excretion, and balances for calcium, phosphorus, and magnesium, calculated in direct proportion to 100 pounds live weight.

The intake of calcium of the sheep on the Russian-thistle hays ranged from 2.174 to 5.066 gm., and all showed negative calcium balances except sheep C–36, which received the smallest amount of calcium. These sheep also showed negative balances for phosphorous on an intake ranging from 0.353 to 0.749 gm. The sheep on the coarse thistles received from 1.977 to 4.288 gm. of magnesium, and all had negative balances except sheep C–36, which received the smallest amount of magnesium. The sheep on the fine thistles, in general, received a little higher level of magnesium, the amounts ranging from 4.334 to 6.545 gm. At this level all had positive balances except sheep J–36.

The three sheep on the ground Russian-thistles with molasses, (trial 39) consumed more feed than the others, and all showed positive balances for calcium, phosphorous, and magnesium.

On the Russian-thistle-alfalfa silage (trial 33) the calcium intake ranged from 9.241 to 12.148 gm., and all the sheep had positive balances. The phosphorous ranged from 0.642 to 0.952 gm., with negative balances for all three sheep. The magnesium ranged from 4.640 to 6.225 gm., and, curiously, sheep I–37, which received the smallest amount of magnesium, showed a positive balance while the other two were negative.

In trial 37, on Russian-thistle-molasses silage, the calcium intake ranged from 15.215 to 21.427 gm. Sheep A–37, on the lower level of calcium, gained 1.039 gm.; whereas, sheep B–37, which received 21.427 gm. of calcium, gained only 0.011 gm, and sheep C–37, on 18.588 gm. of calcium, lost 0.329 gm. Why this group showed such mixed results is not clear. All the sheep in this group had positive phosphorous and magnesium balances.

The sheep on the Russian-thistle-phosphoric acid silage (trial 38), with calcium intakes ranging from 11.332 to 13.678 gm., showed larger positive balances than the sheep in trial 33 on similar intakes of calcium. The phosphorus intake by this group was the highest of any, and two of the sheep showed positive balances. However,

TABLE 13.—*Daily calcium, phosphorus, and magnesium balances of sheep calculated in direct proportion to 100 pounds of live weight*

Trial No.	Sheep No.	Feeds	Calcium Intake in feed and water	Calcium Outgo Feces	Calcium Outgo Urine	Calcium Balance	Phosphorus Intake in feed and water	Phosphorus Outgo Feces	Phosphorus Outgo Urine	Phosphorus Balance	Magnesium Intake in feed and water	Magnesium Outgo Feces	Magnesium Outgo Urine	Magnesium Balance
			Grams	*Grams*	*Gram*	*Grams*	*Grams*	*Grams*	*Gram*	*Gram*	*Grams*	*Grams*	*Grams*	*Gram*
23	A-36	Russian-thistle hay, coarse	3.212	3.175	0.076	-0.039	0.514	0.459	0.099	-0.224	2.944	2.906	0.326	-0.298
	B-36		2.248	4.306	.101	-.159	.681	.057	.039	-.415	3.888	3.382	.607	-.101
	C-36		2.174	2.021	.063	+.090	.353	.202	.575	-.424	1.977	1.531	.321	+.122
	D-36		4.088	4.078	.099	-.089	.655	1.003	.003	-.351	3.746	3.217	.800	-.301
	E-36		4.271	4.268	.075	-.072	.681	.980	.006	-.301	3.923	3.439	.651	-.167
	F-36		4.668	4.694	.088	-.111	.749	.169	.086	-.306	4.288	4.051	.689	-.366
	G-36		5.066	6.358	.077	-1.369	.706	.970	.041	-.305	6.545	6.023	.482	+.010
	H-36		3.951	4.390	.094	-.533	.550	.696	.119	-.265	5.111	4.051	.877	+.183
24	I-36	Russian-thistle hay, fine	3.421	3.464	.073	-.116	.445	.661	.005	-.181	4.560	3.463	.478	+.219
	J-36		3.410	4.108	.059	-.757	.474	.570	.278	-.374	4.419	4.137	.530	-.218
	K-36		3.109	3.633	.074	-.598	.443	.991	.011	-.562	4.334	3.522	.570	+.242
39	G-37	Russian-thistle hay, ground, with molasses	26.115	20.459	.151	+5.505	1.449	.968	.023	+.361	8.768	7.237	.898	+.633
	H-37		23.499	17.608	.138	+5.853	1.294	.912	.018	+.165	7.803	6.618	.727	+.518
33	I-37	Russian-thistle-alfalfa silage	14.247	10.331	.108	+3.808	.783	.608	.011	-.332	4.792	4.020	.883	+.189
	J-37		9.241	8.572	.141	+.525	.642	.919	.025	-.247	4.640	4.161	.276	+.200
	K-37		10.089	10.456	.097	+.136	.838	.918	.167	-.209	5.455	5.361	.680	-.689
37	A-37	Russian-thistle-molasses silage	12.148	11.429	.102	+.617	.952	.605	.556	-.545	6.225	5.622	.817	-.244
	B-37		15.215	14.121	.055	+1.039	1.088	.536	.007	+.083	8.345	7.169	.681	+.495
	C-37		21.427	21.342	.074	+.011	1.531	1.437	.011	+.012	11.673	10.186	1.067	+.420
38	D-37	Russian-thistle-phosphoric acid silage	18.588	18.809	.108	-.329	1.327	1.251	.014	-.138	10.242	8.982	1.009	+.251
	E-37		13.678	10.719	.065	+2.894	2.380	2.509	.009	+.300	7.743	6.529	.634	+.580
	F-37		11.874	10.064	.103	+1.707	2.149	1.334	.515	+.103	6.902	6.495	.272	+.225
			11.332	9.178	.100	+2.054	1.970	1.538	.320		6.434	5.807	.553	+.034

sheep D–37, which received the largest amount of phosphorus of any in the trials, had a negative balance. All three of the sheep in this group had positive magnesium balances.

Mitchell and McClure (*12*) reviewed the mineral requirements of farm animals on the basis of available data and reached the conclusion that ewe lambs weighing from 50 to 110 pounds would require 1.40 gm. of calcium and 1.34 gm. of phosphorus and that ram lambs weighing from 50 to 120 pounds would require 1.59 gm. of calcium and 1.48 gm. of phosphorus. No requirements for magnesium were given.

If the figures of Mitchell and McClure are used as a basis for comparison, it is evident that all the sheep received considerably more calcium than the indicated requirements; yet, a considerable number of negative balances were observed. In trial 33, where the calcium intake ranged from 9.241 to 12.148 gm., small positive balances were obtained, but in trial 38, where the calcium intakes ranged from 11.332 to 13.678 gm., the positive balances were notably higher. This suggests a possible difference in the availability of the calcium in the two feeds. In the series as a whole, with one exception, positive calcium balances were obtained only where more than 10 gm. of calcium were fed per hundred pounds of live weight.

Comparing the phosphorus balances in the same way, it is observed that, where more than 1 gm. of phosphorus was consumed, the balances were positive, except for the erratic result from sheep D–37 in trial 38. This would indicate that the requirements for phosphorus suggested by Mitchell and McClure should be adequate.

A consideration of the magnesium balances shows 8 negative balances out of 23 trials. Two sheep, J–37 on 5.455 gm. and K–37 on 6.225 gm. of magnesium, showed negative balances, but the other 6 negative balances were on intakes of less than 4.640 gm. of magnesium. On intakes from 4.640 gm. to 11.673 gm. of magnesium, the balances were all positive—except as noted. From the data obtained, it appears that approximately 4.5–5 gm. daily intake of magnesium per 100 pounds of live weight was needed to meet the maintenance requirements of lambs under the above-described conditions. The low digestibility of the Russian-thistle possibly accounts for the poor utilization of the magnesium present in the ration.

It is evident that calcium, phosphorus, and magnesium may be stored by sheep on rations that are below the maintenance requirements in protein and total digestible nutrients or energy. How much, if at all, the intakes of these elements might be lowered and still give positive balances on rations adequate in protein and energy is not indicated by these trials.

DISCUSSION OF RESULTS

The Russian-thistle has been used for years in the Great Plains area as an emergency feed, particularly for cattle. By its use farmers have been enabled to keep livestock through winter seasons when otherwise they would have had to sell off stock or suffer heavy losses because of an inadequate feed supply. The composition of the thistles and the

results of the metabolism trials show that the thistles compare favorably with red clover hay in digestible protein and that they furnish nearly as much digestible nutrients as oat straw and more than wheat straw on the basis of Morrison's (*13*) tables. According to Morrison, oat straw on a dry basis contains 49.2 and wheat straw 39.6 percent of total digestible nutrients, as compared to 44.3 percent in the coarse" thistles and 46.7 percent in the "fine" thistles.

It is evident, therefore, that, if enough is eaten, and if the thistles have no injurious effects on the animals, Russian-thistles should prove a valuable feed in times of emergency. The writers' experience is that the cured thistles are unpalatable to sheep and that moistening them with water alone is not sufficient to induce the sheep to eat enough for maintenance. Moistening the thistles with water and cane molasses induced the sheep to consume enough for maintenance, as is shown in trial 39. This, then, is one method of inducing sheep to eat more of the thistles. The silages appeared to be somewhat more palatable than the hays; but, even so, less was consumed than is required for maintenance. Evidently, the thistles should be used as a part of the ration and not as the sole feed.

Russian-thistles usually are laxative in their effect, sometimes excessively so, but in these trials the feces were only soft and pasty. To avoid undue laxative effects, cane molasses was used with the thistles, rather than beet molasses.

No injurious effects from feeding Russian-thistles to sheep have been observed in these trials or in feeding the thistles to bred ewes during four winters.

SUMMARY

Metabolism trials with sheep were conducted in which the composition and digestibility of Russian-thistles (*Salsola kali*) were determined as hay and as silage. The trials included determinations of metabolizable energy and nitrogen, calcium, phosphorus, and magnesium balances.

Six individual metabolism trials on a rather coarse Russian-thistle hay indicated, on a dry basis, 8.5 percent digestible protein, 44.3 percent total digestible nutrients, and 68.44 therms of metabolizable energy per 100 pounds. Five individual trials on a finer thistle hay indicated 10.1 percent digestible protein, 46.7 percent total digestible nutrients, and 67.88 therms of metabolizable energy per 100 pounds.

Three individual trials on ground Russian-thistles with molasses, on a dry basis, indicated 8.2 percent digestible protein, 53.8 percent total digestible nutrients, and 78.03 therms of metabolizable energy per 100 pounds.

Three individual trials on Russian-thistle silage, which included some alfalfa and which had been stored about 2½ years, contained, on a dry basis, 5.3 percent digestible protein, 38.8 percent total digestible nutrients, and 66.08 therms of metabolizable energy per 100 pounds.

Three individual trials on a Russian-thistle-molasses silage, made from chopped fine thistles by adding cane molasses and water, contained, on a dry basis, 9.2 percent digestible protein, 40.5 percent of total digestible nutrients, and 65.46 therms of metabolizable energy

per 100 pounds. This indicates a reduction in the digestible protein, total digestible nutrients, and metabolizable energy as compared to the hay (trial 24).

Three individual trials cn silage made from the same lot of hay as the thistle-molasses silage, but preserved with phosphoric acid, contained on a dry basis, 8.3 percent digestible protein, 37.7 percent total digestible nutrients, and 54.26 therms of metabolizable energy per 100 pounds. These values are lower than the values on the same hay preserved with molasses.

The metabolizable energy per pound of digestible organic matter, in all trials, ranged from 1.465 to 1.796, with an average of 1.614 therms.

The metabolizable energy per pound of total digestible nutrients, in all trials, ranged from 1.379 to 1.728, with an average of 1.516 therms.

With one exception, positive calcium balances were observed only in the trials where more than 10 gm. of calcium were fed per 100 pounds live weight.

Positive balances for phosphorus were observed when more than 1 gm. of phosphorus was consumed per 100 pounds live weight, except in two trials.

The data indicate than an abnormally high intake of 4.5 to 5.0 gm. of magnesium daily was required to keep the animal in positive magnesium balance. The low digestibility of the ration may account for these high magnesium intakes.

LITERATURE CITED

(1) ARMSBY, H. P.
    1917. THE NUTRITION OF FARM ANIMALS. 743 pp., illus. New York.
(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
    1940. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 5, 757 pp., illus. Washington, D. C.
(3) BRIGGS, L. J., and SHANTZ, H. L.
    1914. RELATIVE WATER REQUIREMENT OF PLANTS. Jour. Agr. Res. 3: 1–64, illus.
(4) BRODY, S.; PROCTER, R. C., and ASHWORTH, U. S.
    1934. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO DOMESTIC ANIMALS. XXXIV. BASAL METABOLISM, ENDOGENOUS NITROGEN, CREATININE AND NEUTRAL SULPHUR EXCRETIONS AS FUNCTIONS OF BODY WEIGHT. Mo. Agr. Expt. Sta. Res. Bul. 220, 40 pp., illus.
(5) CAVE, H. W., RIDDELL, W. H., and HUGHES, J. S.
    1936. THE DIGESTIBILITY AND FEEDING VALUE OF RUSSIAN THISTLE HAY. Jour. Dairy Sci. 19: 285–290.
(6) CHRISTENSEN, F. W., and HOPPER, T. H.
    1932. EFFECT OF WEATHERING AND STAGE OF MATURITY ON THE PALATABILITY AND NUTRITIVE VALUE OF PRAIRIE HAY. N. Dak. Agr. Expt. Sta. Bul. (Tech.) 260, 55 pp., illus.
(7) ——— and HOPPER, T. H.
    1938. DIGESTIBLE NUTRIENTS AND METABOLIZABLE ENERGY IN CERTAIN SILAGES, HAYS, AND MIXED RATIONS. Jour. Agr. Res. 57: 477–512.
(8) DILLMAN, A. C.
    1931. THE WATER REQUIREMENT OF CERTAIN CROP PLANTS AND WEEDS IN THE NORTHERN GREAT PLAINS. Jour. Agr. Res. 42: 187–238, illus.
(9) HOPPER, T. H.
    1930. A RAPID BULK-SAMPLE DRIER. Indus. and Engin. Chem., Analyt. Ed. 2: 198, illus.

(10) KRISS, M.
　　　1931.   A COMPARISON OF FEEDING STANDARDS FOR DAIRY COWS, WITH
　　　　　　　ESPECIAL REFERENCE TO ENERGY REQUIREMENTS.   Jour. Nutr.
　　　　　　　4: 141–161.
(11) MITCHELL, H. H., KAMMLADE, W. G., and HAMILTON, T. S.
　　　1928.   RELATIVE ENERGY VALUE OF ALFALFA, CLOVER, AND TIMOTHY HAY
　　　　　　　FOR THE MAINTENANCE OF SHEEP.   Ill. Agr. Expt. Sta. Bul.
　　　　　　　317, 167 pp., illus.
(12) ——— and McCLURE, F. J.
　　　1937.   MINERAL NUTRITION OF FARM ANIMALS.   Natl. Res. Council Bul.
　　　　　　　99, 135 pp.
(13) MORRISON, F. B.
　　　1936.   FEEDS AND FEEDING . . . Ed. 20, 1050 pp., illus.   Ithaca, N. Y.
(14) STEVENS, O. A.
　　　1943.   RUSSIAN THISTLE LIFE HISTORY AND GROWTH.   N. Dak. Agr. Expt.
　　　　　　　Sta. Bul. 326, 20 pp., illus.

RECOVERY AND VIABILITY OF SEEDS OF CERTAIN SOUTHERN GRASSES AND LESPEDEZA PASSED THROUGH THE BOVINE DIGESTIVE TRACT [1]

By GLENN W. BURTON, *senior geneticist, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering,* and JOHN S. ANDREWS, *parasitologist, Zoological Division, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

The role that domestic animals play in the dissemination of weed seeds has been the subject of study since early in the present century, but many questions are still unanswered. A number of people have expressed the opinion that such southern forage plants as carpet, Bermuda, and Johnson grasses are disseminated by cattle, but a thorough search of the literature has revealed no publications dealing with the recovery and viability of such seeds after passage through the bovine digestive tract. In 1942 and 1945 studies were therefore made to learn what proportion of the seeds of certain southern grasses and lespedeza is digested by Jersey cows and what proportion passes through the digestive tract and still remains viable. The experiments were designed to answer the following questions: Are cattle effective agents in the dissemination of grass seeds? Have they been responsible for the spread of undesirable grasses? How many days are required to clear the bovine digestive tract of viable seeds of the various grasses and lespedeza? What percentage of the seeds of southern grasses and lespedeza consumed are digested and presumably utilized by the cattle for maintenance and growth? The results of the experiments are reported herein.

REVIEW OF LITERATURE

As early as 1907 Hills and Jones (7) [3] recognized that commercial feedstuffs contain viable weed seeds that cattle and horses do not digest. Hills, Jones, and Benedict (8) continued the studies.

In 1911 Korsmo (9) studied this problem by feeding the seeds of six different weeds to horses, cattle, and hogs. He found that seeds which had passed through the digestive tract of cattle germinated better than those of the same species recovered in the feces of horses and hogs.

Beach (2) reported that 4.5 percent of weed seeds having a viability of 26.4 percent germinated after passing through the digestive tract of a Jersey cow.

[3] Italic numbers in parentheses refer to Literature Cited, p. 103.

In India, Milne *(10)* observed that 9.6 to 20.5 percent of the wheat seeds passed by cattle produced plants.

Atkeson, Hulbert, and Warren *(1)* fed dairy cows 1 quart of weed seeds night and morning and recovered the first seeds 47 hours after they were fed. In the 19 samples representing 13 different species studied during the 2 trials, reduction in germination due to digestion was more than 90 percent in 6 samples and 80 percent or more in 11 samples. The writers concluded that the longer the seeds remained in the digestive tract the greater the reduction in germination.

Known quantities of seven weed seeds were fed to calves, horses, sheep, hogs, and chickens by Harmon and Keim *(5)*. All of the feces were recovered for 4 days. During this period 23.1 percent of the weed seeds fed to the calves were recovered, and these had an average viability of 45.2 percent. This was the only experiment reviewed in which an effort was made to determine the percentage of seeds recovered.

Dore and Raymond *(3)*, using flats in the greenhouse, germinated seeds found in manure droppings from old pastures in southern Quebec. In 6 samples of manure they found 49 species with 167 to 970 seeds per 10 ounces of dry manure. *Agrostis stolonifera* L., *Phleum pratense* L., *Poa pratensis* L., and *Trifolium repens* L. exceeded the other species in the number of viable seeds per unit of manure.

MATERIAL AND METHODS

On October 3, 1942, three mature Jersey cows were placed in separate cement-floored box stalls. From this date throughout the experiment, except as indicated for October 10, these animals were fed a diet known to be free of the seeds of the grasses and the lespedeza (legume) to be studied. An examination of the feces of these cows on October 9 revealed that only a very few seeds of carpet and Bermuda grasses were being passed. Therefore, on October 10, two of the cows were each fed the following seed mixture: Common Bahia grass (*Paspalum notatum* Flügge), 675 gm.; Paraguay Bahia grass (*P. notatum*), 340 gm.; Pensacola Bahia grass (*P. notatum*), 225 gm.; Dallis grass (*P. dilatatum* Poir.), 450 gm.; Johnson grass (*Sorghum halepense* (L.) Pers.), 900 gm.; carpet grass (*Axonopus affinis* Chase), 40 gm.; Bermuda grass (*Cynodon dactylon* (L.) Pers.), 40 gm.; and an unnamed strain of common lespedeza (*Lespedeza striata* (Thunb.) H. and A.) in the hull, 900 gm. The animals consumed an estimated 90 percent of this mixture.

All feces dropped by each animal during each 24-hour period were collected and thoroughly mixed; 200- and 500-gm. samples were taken in duplicate and carefully washed through a series of screens that would retain all the seeds. The material that did not pass through the screens was dried. Seed-cleaning equipment was used to separate the seeds; the number of each species in each 200-gm. sample was counted to permit a calculation of the number of seeds passed during each 24-hour period (table 1). A few seeds of carpet and Bermuda grasses were passed for several days by the third cow, which was fed no seeds on October 10. It was assumed that the cows which were fed seeds passed a similar number of seeds that had been eaten before the experiment was begun and corrections were made in the counts

reported. One of the duplicate 500-gm. samples collected during a 24-hour period was spread out about one-half inch thick on bread pans containing steam-sterilized soil. After all soluble and fine material was washed from the other sample, it was planted in similar pans of sterilized soil. These cultures were watered daily and were kept in a greenhouse heated to temperatures that would favor the germination of the seeds planted. The seedlings of each species in each pan were counted, recorded, and removed at weekly intervals. Such counts for a 12-week period furnished the basis for calculating the number of viable seeds of each species recovered from each cow (table 2).

The caryopses (grains or fruits of grasses) of Dallis, carpet, Bahia, and Bermuda grasses as sold on the market are usually enclosed very tightly in glumes. In such commercial seed lots frequently occur many pairs of glumes that do not contain caryopses because it is difficult to separate them in the cleaning process. These cannot be distinguished from good seeds by outward appearance. In the experiment just described the actual number of caryopses in the seed fed was not determined. The number of seedlings that a known weight of the commercial seed would produce was ascertained by germinating duplicate 100-seed samples in steam-sterilized soil at the same time that the other germination trials were run. From this information it was possible to calculate the number of viable seeds of each species fed (table 2).

In an effort to measure more accurately the influence of passage through the bovine digestive tract on the viability of the seeds of southern grasses and lespedeza, a second experiment was conducted in 1945. Since mature cows were not available, 2-year-old Jersey heifers were used. Three heifers were placed in box stalls with concrete floors and fed a ration free of grass and lespedeza seeds until they were no longer passing seeds of any of the species to be studied. On March 10, 1945, the quantities and numbers of seeds or caryopses shown in table 3 were fed to each heifer. Kobe lespedeza (*Lespedeza striata*) was used instead of common lespedeza in this study. During each 24-hour interval for 10 days, beginning March 11, all feces were collected from each animal. All seeds in each lot of feces were then recovered by the techniques previously described. The number of seeds of each species recovered each day was determined (table 4). Duplicate 100-seed samples of each species were then counted from each lot of seed passed by each heifer during each 24-hour period. In the collections made toward the end of the study there were not enough seeds to give two 100-seed samples. In such cases all of the seeds recovered were counted and used. All 100-seed samples were planted in steam-sterilized soil in the greenhouse and were allowed to germinate for 6 weeks under conditions of optimum temperature and moisture (table 5). Duplicate 100-seed samples of the original seed lots were included in these viability tests as checks. All data from both of these studies were subjected to statistical analyses. The analyses of the 1945 experiment are presented in table 6.

The word "seeds" as used in describing the results of these experiments refers to the true lespedeza seeds and also the caryopses of the grasses, which are usually enclosed in glumes.

RESULTS

The majority of the viable seeds recovered in 1942 were passed during the second day (table 1). It is significant that a few viable seeds of each species were still being passed 10 days after they were fed. However, the quantities of viable seeds recovered on the ninth and tenth days were so small as to suggest that practically all the other viable seeds fed on October 10 had been destroyed by mastication and digestion. Of considerable interest was the observation that the feces of one of the cows, although normal in quantity, contained no seeds alive or dead on the third day. This cow passed many viable seeds on the second and fourth days, however.

The percentage recovery of viable seeds fed to mature Jersey cows was not the same for all species (table 2). Over half of the viable Bermuda grass seeds fed to these cows passed through their digestive tracts and retained their viability. Rather high percentages of living seeds of carpet grass and Pensacola Bahia grass were recovered.

TABLE 1.—*Daily recovery of seeds of several southern grasses and common lespedeza from feces of 2 mature Jersey cows during the 10 days after feeding, October 1942*

Species	Portion of total seeds recovered on day indicated									
	1	2	3[1]	4	5	6	7	8	9	10
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Common Bahia grass	3.3	57.3	6.1	19.2	5.7	2.3	2.6	2.5	0.4	0.1
Paraguay Bahia grass	2.3	51.2	6.2	23.2	8.4	2.9	3.2	1.7	.5	.1
Pensacola Bahia grass	3.2	48.0	10.0	24.4	7.9	2 4	2.1	1.4	.2	.1
Johnson grass	5.4	70.3	8.6	9.5	2.5	.7	2.0	.4	.2	Trace
Dallis grass	4.1	62.0	6.3	20.3	3.3	2.1	1.3	.3	.1	Do.
Carpet grass	5 1	72.9	5.0	12.8	1.6	.8	1 0	.2	.3	.2
Bermuda grass	5.9	60.4	10.0	16 4	3.5	1.7	.9	.7	.1	Trace
Common lespedeza	12 2	72.8	5.1	6.5	1.5	.4	.7	.3	.1	Do.
Average	5.2	61.9	7.2	16.5	4.3	1.7	1.7	.9	.2	.1

[1] Lower than the fourth day because 1 cow passed no seeds.

TABLE 2.—*Viability of seeds of several southern grasses and common lespedeza fed to 2 mature Jersey cows and collected during the 10 days after feeding, October 1942*

[Viability based on total seedlings that appeared in steam-sterilized soil in 12 weeks under optimum temperature and moisture conditions in the greenhouse]

Species	Viable seeds fed [1]	Seedlings from seeds found in feces recovered in 10 days		Viable seeds germinated	
		Washed feces [2]	Unwashed feces	Washed feces [2]	Unwashed feces
	Number	Number	Number	Percent	Percent
Common Bahia grass	2,060	42	4	2.0	0.2
Paraguay Bahia grass	10,050	1,520	593	15.1	5.9
Pensacola Bahia grass	35,695	14,851	4,879	41.6	13.7
Johnson grass	82,510	312	1,507	.4	1.8
Dallis grass	81,095	1,055	277	1.3	.3
Carpet grass	48,600	14,200	7,732	29.2	15.9
Bermuda grass	59,985	31,368	21,897	52.3	36.5
Common lespedeza	315,525	1,577	329	.5	.1

[1] Calculated from viability and weight of seed fed.
[2] All material finer than the smallest seeds was washed from the feces.

On the other hand, less than 2 percent of the viable seeds of Dallis grass, Johnson grass, and common lespedeza ingested retained their viability after passing through the cows. It is important to note that some viable seeds of all species were recovered, since some may be seeds of serious weeds. Viable seeds of all species were found in washed feces collected up to 7 days after the seeds were fed to the cows. Viable Bermuda grass seeds were still being passed at the end of 10 days.

In most instances the seeds in washed feces germinated better than those in unwashed, perhaps because of the higher salt concentrations in the unwashed feces. Seeds of Johnson and Bermuda grasses seemed relatively better able to germinate in unwashed feces than those of the other species included in this test.

In the first experiment all records were based on the viability of the seeds fed and recovered. This test failed to show whether the loss in viability was due to the destruction of the seeds by mastication and digestion or was a result of the digestive juices acting upon the seeds without causing external change. It obviously failed to indicate the quantities of seed that were digested. To obtain this information, the actual numbers of seeds and caryopses fed and recovered were determined in a second experiment, conducted in 1945 (table 3). These data show that approximately half of the Bahia and Bermuda grass seeds were recovered, while less than 12 percent of the lespedeza seeds passed intact through the digestive tract.

TABLE 3.—*Seeds of several southern grasses and Kobe lespedeza fed to and recovered from 3 Jersey heifers, March 1945*

Species	Seeds fed (Mar. 10)		Seeds re-covered (Mar. 11–20)
	Grams	*Number*	*Number*
Common Bahia grass	330	130, 260	61, 950
Pensacola Bahia grass	175	176, 770	93, 460
Johnson grass	440	251, 420	82, 440
Bermuda grass	40	307, 690	145, 260
Carpet grass	60	248, 570	82, 160
Dallis grass	250	208, 660	47, 470
Kobe lespedeza	150	121, 460	14, 405

The daily recovery of southern grass and lespedeza seeds passed through the digestive tracts of the three heifers used in this experiment is summarized in table 4 and statistically analyzed in table 6. The analyses show that the cows passed significantly different quantities of seeds, that some species passed through the digestive tract more successfully than others, and that the quantities recovered from day to day differed significantly. The cows × days interaction was highly significant because the seeds did not pass through each cow with the same speed. The highly significant species × days interaction proves that not all the species passed through the digestive tract at the same rate. Table 4 shows that the seeds of the small-seeded grasses like Bermuda and carpet grasses passed through the digestive tract faster than those of the large-seeded species such as Johnson and Bahia grasses.

Since there was a gradual decline in the number of seeds recovered after the fifth day and in most cases less than 1 percent of the seeds

were passed on the tenth day, it is safe to conclude that very few seeds would have been passed later. It is logical to assume that most of the seeds not recovered were digested and contributed to the nutritional needs of the animals. If this assumption is correct, the data in table 4 would indicate that 88 percent of the lespedeza seeds, 77 percent of the Dallis grass seeds, 67 percent of the carpet and Johnson grass seeds, and approximately 50 percent of the Bermuda and Bahia grass seeds were digested. These percentages are inversely proportional to the hardness of the seeds and their protective coverings.

TABLE 4.—*Seeds of several southern grasses and Kobe lespedeza recovered daily in feces of 3 Jersey heifers during the 10 days after feeding, March 1945*

Species	Seeds recovered per 10,000 fed on day No.—										Total
	1	2	3	4	5	6	7	8	9	10	
Common Bahia grass	174	795	1,043	492	993	687	339	129	69	35	4,756
Pensacola Bahia grass	155	897	1,228	607	972	700	465	163	62	38	5,287
Johnson grass	92	639	780	379	558	441	230	112	32	16	3,279
Bermuda grass	190	1,286	1,271	585	583	428	249	71	39	19	4,721
Carpet grass	159	769	916	346	416	395	185	62	40	16	3,304
Dallis grass	62	282	506	327	412	313	189	119	43	22	2,275
Kobe lespedeza	64	284	361	100	166	84	81	21	12	13	1,186

The data in table 5 show the influence of time spent in the digestive tract upon the viability of the seeds that passed through it. The statistical analyses of the original data from which this table was summarized appear in table 6. If these tables are considered together it is evident that passage through the digestive tract did not affect all seeds in the same way. Although the seeds of most of the species lost some of their viability as a result of passing through the digestive tract, Bermuda grass seeds actually germinated better for having been subjected to the digestive processes for a period up to 6 days after the seeds were fed.

In general, the longer the seeds remained in the digestive tract the lower their viability upon recovery. It is evident that some species lost their viability more rapidly than others in the digestive tract. Table 6 shows that this species $\times$ days interaction was highly significant. The Johnson grass seeds used in this experiment were old and low in viability. Since Johnson grass seeds in the first experiment germinated after 7 days in the digestive tract, it seems very probable that the viability results obtained from the second test were influenced by the poor quality of the seeds used. It seems logical to suppose that fresh seeds could retain their viability under adverse conditions for a longer period than old ones.

The highly significant cows $\times$ species interaction permits some interesting speculation. Bermuda grass seeds recovered from cow 3 during the 10 days of the test averaged 13 percent higher in germination than Bermuda grass seeds from cow 1 or 2. Since these seeds were germinated in the same flat under carefully controlled conditions, the differences could not be credited to the germination technique. Seeds of the other species lost as much of their viability in passing through cow 3 as through the other cows in the test. Evidently the digestive processes of the heifers in this test differed enough to have a differential effect upon the viability of the ingested seeds of certain species.

TABLE 5.—*Viability of seeds of several southern grasses and Kobe lespedeza recovered daily in feces of 3 Jersey heifers during the 10 days after feeding, March 1945*

[Seeds planted in steam-sterilized soil and allowed to germinate for 6 weeks under optimum temperature and moisture conditions in the greenhouse]

Species	Seeds germinating before being fed	Seeds germinating after being recovered on day No.—									
		1	2	3	4	5	6	7	8	9	10
	Per-cent	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*	*Per-cent*
Common Bahia grass	19	19	15	11	9	6	4	2	1	1	0
Common Bahia grass (scarified)	38	37	29	21	14	11	9	2	1	1	2
Pensacola Bahia grass	73	37	39	35	25	15	12	6	2	5	1
Pensacola Bahia grass (scarified)	77	46	50	43	34	22	16	14	4	3	3
Johnson grass	17	10	4	1	0	0	0	0	0	0	0
Bermuda grass	62	78	85	83	82	79	70	61	51	51	46
Carpet grass	43	68	57	34	28	19	14	3	7	12	1
Dallis grass	34	47	35	12	6	2	0	0	1	0	0
Kobe lespedeza	20	5	7	7	9	12	10	9	7	8	2

TABLE 6.—*Analyses of variance of recovery and viability of seeds of several southern grasses and Kobe lespedeza passed through the digestive tracts of 3 Jersey heifers, March 1945*

Source of variation	Degrees of freedom	Mean square of number of seeds recovered per 10,000 fed	Mean square of percentage of seeds germinating
Cows	2	184, 645**	78
Species	6	660, 816**	15, 709**
Days	9	1, 850, 892**	2, 339**
Cows×species	12	15, 333	121**
Cows×days	18	227, 963**	67
Species×days	54	71, 372**	265**
Error	108	11, 698	35

** *F* exceeds the 1-percent level of significance.

DISCUSSION

The number of days required to clear grass seeds from the digestive tract of a cow is of considerable practical importance. Particularly is this true if the seeds consumed are those of weeds that are difficult to control. Cattle have been charged with the spreading of Johnson and Bermuda grasses. Lawsuits have even been filed against individuals who sold cattle that infested the buyers' fields with Johnson grass seeds.

It is evident from the results of the foregoing experiments that cattle will pass viable seeds of all the species studied for at least a week after they have been removed from the source of the seeds. In order to prevent cattle from carrying seeds of such plants from one field to another, it will be necessary to remove them from the source and give them feed known to be free of the seeds for at least 10 days to 2 weeks.

Carpet grass, an introduced species, is now widely distributed in the flatwoods of Georgia and Florida. Since man rarely seeded it in these sections the dissemination of the seeds cannot be credited directly to him. It is generally believed that cattle have been the principal agents of dissemination. The results of the experiments reported herein indicate that seeds of the forage grasses which will probably be

used in the piney-woods sections for pasture improvement can pass
through the digestive tracts of cows and germinate in the feces. How
effective cows may be as agents in disseminating the seeds and estab-
lishing the new area can only be suggested at this time. The length
of time during which the grasses produce viable seeds, the palatability
of the seeds and seedstalks, the range of the animals consuming the
seeds, the number of defecations of the animals, and the location of
salt, water, and shade are all factors that will influence the efficacy of
cows in establishing a new grass. Bahia grass, for example, produces
viable seeds continuously for approximately 150 days during the year.
Dukes (4) reported that dairy cows defecate 10 to 24 times daily.
During a single seed-producing period one cow might be expected to
defecate from 1,500 to 3,000 times, each dropping carrying viable
Bahia grass seeds. The presence of the plant food in the feces should
help to insure the establishment of the grass in each dropping. In the
cut-over piney woods where water and shade are naturally scattered,
a good random distribution of the droppings should result from exer-
cising some care in moving salt boxes. Widely separated strips of the
Bahia grass established by man at very little cost could serve as a seed
source for the cattle. It is conceivable that man may come to recog-
nize the cow as an effective partner in reseeding the cut-over piney
woods of the Southeast.

If it is correct to assume that the seeds not recovered in this experi-
ment were digested, it may be concluded that the seeds of the forage
plants considered here do, as suggested in the introduction, contribute
to the nutrition of the cattle consuming them. These experiments
suggest, for example, that 88 percent of the lespedeza seeds consumed
were digested. Yields of 500 to 600 pounds of lespedeza seed per
acre are not uncommon. Herman and Ragsdale (6) compared finely
ground Korean lespedeza seed with a mixture of cottonseed and
soybean meals as the main source of protein for lactating cows.
They concluded that the proteins of ground Korean lespedeza seed
were equal pound for pound to the proteins of a mixture of equal parts
of cottonseed and soybean meals in the ration of lactating cows.
Perhaps this helps to explain why cattle fatten well when grazing
lespedeza in the late summer and fall.

SUMMARY

Known quantities of seeds of common, Paraguay, and Pensacola
Bahia grasses, Johnson grass, Dallis grass, carpet grass, Bermuda
grass, and common or Kobe lespedeza were fed to mature cows and
heifers. The seeds of each species recovered at daily intervals and
the viability of the seeds recovered were determined in two experi-
ments.

The majority of the seeds fed were recovered in the second and third
days, but a few seeds of each species were still being passed 10 days
after they were fed.

In most instances the seeds germinated better in washed feces than
in unwashed. Johnson grass and Bermuda grass seeds germinated
relatively better in unwashed feces than the other species studied.

Statistical analyses of the results revealed that cows that were fed the same quantities of seed passed significantly different amounts, that seeds of some species passed through the digestive tract more successfully than others, that the quantities of seed recovered from day to day differed significantly, and that the seeds of all species did not pass through the digestive tract with the same speed. The seeds of the small-seeded grasses passed through the digestive tract faster than those of the large-seeded grasses.

Approximately one-half of the Bahia and Bermuda grass seeds fed, one-third of the carpet and Johnson grass seeds fed, one-fourth of the Dallis grass seeds fed, and one-eighth of the lespedeza seeds fed were recovered in a 10-day period. The evidence presented indicates that most of the seeds not digested were passed within 10 days after the seeds were fed.

Passage through the digestive tract reduced the viability of the seeds of most species in varying degrees but actually induced a greater number of Bermuda grass seeds to germinate.

In general, the longer the seeds remained in the digestive tract the lower their viability upon recovery. Seeds of some species lost their viability more rapidly than others.

LITERATURE CITED

(1) ATKESON, F. W., HULBERT, H. W., and WARREN, T. R.
   1934. EFFECT OF BOVINE DIGESTION AND OF MANURE STORAGE ON THE VIABILITY OF WEED SEEDS. Amer. Soc. Agron. Jour. 26: 390–397.
(2) BEACH, C. L.
   1909. VIABILITY OF WEED SEEDS IN FEEDING STUFFS. Vt. Agr. Expt. Sta. Bul. 138, pp. [11]–20.
(3) DORE, W. G., and RAYMOND, L. C.
   1942. PASTURE STUDIES. XXIV. VIABLE SEEDS IN PASTURE SOIL AND MANURE. Sci. Agr. 23: 69–79, illus.
(4) DUKES, H. H.
   1942. THE PHYSIOLOGY OF DOMESTIC ANIMALS. Ed. 5, rev., 721 pp., illus. Ithaca, N. Y.
(5) HARMON, G. W., and KEIM, F. D.
   1934. THE PERCENTAGE AND VIABILITY OF WEED SEEDS RECOVERED IN THE FECES OF FARM ANIMALS AND THEIR LONGEVITY WHEN BURIED IN MANURE. Amer. Soc. Agron. Jour. 26: 762–767.
(6) HERMAN, H. A., and RAGSDALE, A. C.
   1942. THE FEEDING OF KOREAN LESPEDEZA SEED AS A PROTEIN SUPPLEMENT FOR MILK PRODUCTION. (Abstract) Jour. Dairy Sci. 25: 681–682.
(7) HILLS, J. L., and JONES, C. H.
   1907. COMMERCIAL FEEDING STUFFS. Vt. Agr. Expt. Sta. Bul. 131, 8 pp., illus.
(8) ——— JONES, C. H., and BENEDICT, P. A.
   1910. COMMERCIAL FEEDING STUFFS. PRINCIPLES AND PRACTICE OF STOCK FEEDING. Vt. Agr. Expt. Sta. Bul. 152, pp. [513]–614.
(9) KORSMO, E.
   1911. THE VIABILITY OF WEED SEEDS AFTER PASSING THROUGH THE DIGESTIVE TRACT OF FARM ANIMALS. Tidsskr. for det Norske Landbr. 18: 223–230. [In Norwegian. English title from [U. S.] Expt. Sta. Rec. 26: 839. 1912.]
(10) MILNE, D.
   1915. VITALITY OF SEEDS PASSED BY CATTLE. Agr. Jour. India 10: 353–369.

YIELD, COMPOSITION, AND OTHER LATEX CHARACTERISTICS OF CRYPTOSTEGIA GRANDIFLORA[1]

By W. S. STEWART, formerly *plant physiologist, Division of Rubber Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture;* J. BONNER, *professor of plant physiology, California Institute of Technology;* and R. W. HUMMER, formerly *associate chemist, Division of Rubber Plant Investigations* [2]

INTRODUCTION

Cryptostegia grandiflora R. Br., a member of the Asclepiadaceae, has long been known as a producer of rubber. This climbing shrub, which is tolerant of a wide diversity of soils and climates, grows as an escape in tropical and subtropical parts of Mexico and Central America as well as under cultivation in southern Florida, Texas, and California. It was a sufficiently promising war-emergency source of rubber to encourage further investigation. This paper presents results of some of the studies made by the United States Cryptostegia Research Laboratory, Ciudad Victoria, Tamaulipas, Mexico, during 1942–45.[3]

Cryptostegia grandiflora, as well as the related *C. madagascariensis* Boj., was described by Polhamus, Hill, and Elder (*12*),[4] and the morphology and anatomy of *C. grandiflora* were studied in detail by Artschwager (*3*). The plant grows rapidly from seed and produces a rounded shrub 6 feet or more in height. In addition to the usual foliage stems, long, upright, unbranched leaders 10 to 20 feet in length known as whips are produced in quantity. The whips will climb if a support is available, but in the absence of a support they ultimately bend over because of their own increasing weight and produce leafy lateral branches that are incorporated into the body of the plant.

Rubber from *Cryptostegia grandiflora* is obtained principally by bleeding the latex from the whips. The latex is contained in latex vessels found in the stems, roots, and leaves and even in the fruits. In the whips the latex vessels are present in the pith and the bark. Those of the pith, which number 300 to 800 and average about 25μ

Journal of Agricultural Research,   Vol. 76, Nos. 5 & 6
Washington, D. C.   March 1, 15, 1948
         Key No. G–1390

(105)

in diameter, are formed early in the development of the whip and do not increase in number as the whip matures. The vessels of the bark, on the contrary, increase in number during the course of secondary growth. According to Whittenberger, Brice, and Copley (*19*), the proportionate amount of rubber in the bark increases as the whip matures. Thus, in whips 3 to 4 mm. in diameter 75 percent of the rubber was contained in the pith, whereas in those 17 mm. in diameter 74 percent was found in the bark.

MATERIALS AND METHODS

The plants used in these investigations were grown in the field at the laboratory experimental farm at Ciudad Victoria and at the experimental farm of the Mexican Government at Llera, Tamaulipas, Mexico. The seeds were germinated in nursery beds, and the seedlings were transplanted to the field when they had attained a height of 10 to 15 inches. Generally the plants were pruned to a height of 6 inches when transplanted and were planted in 3-acre plots in one of several spacings (1 by 1 to 3 by 12 feet). Cultural practices were then followed to maintain the plants in thrifty condition. An additional planting of about 3 acres of *Cryptostegia grandiflora* near Matamoros, Tamaulipas, Mexico, was also made available through the kindness of Ing. Eduardo Chavez. These plants were between 1 and 2 years old in 1943 and were growing in various spacings. They were irrigated as necessary and maintained in good growth.

Latex was usually obtained by whip bleeding. It was collected in cylindrical glass bottles about 3 cm. in diameter and 6 cm. in height. These were fitted with metal-rim screw caps which secured a rubber diaphragm over the top of the bottle. A hole 3 mm. in diameter allowed for the insertion of a cut whip through the diaphragm into the bottle. When successive bleedings of the same whip were made, the small plug of coagulated latex forming on the surface of the cut was removed by hand and weighed. This coagulate is referred to as "plug rubber," or "stem coagulate." "Total coagulate" includes the plug rubber as well as latex rubber. Latex coagulated by adding an equal volume of 95-percent ethyl alcohol is referred to as "coagulate," or "alcohol coagulate." For most yield studies the latex was alcohol-coagulated. Latex that was dried without addition of a coagulant is referred to as "total solids," or "latex total solids." The latex total solids were usually used in studies of a theoretical nature.

LATEX FLOW AND EXUDATION

FORCES INVOLVED IN LATEX FLOW

The force causing latex flow and exudation in *Hevea* was discussed by Arisz (*2*), Frey-Wyssling (*6, 7*), Van Iterson (*10*), and others, and the literature on the subject was reviewed by Moyer (*11*). In general it may be stated that a latex vessel in an intact stem is under turgor pressure; the latex, for example, is under hydrostatic pressure. When a bleeding cut is made, the turgor pressure is reduced to zero at the point of incision while at a point some distance from the incision the original pressure still exists. The gradient in hydrostatic pressure thus set up on the latex is the driving force that causes latex flow and exudation from an incision. The force involved in latex flow is of

osmotic origin; the maximum pressure under which latex may be exuded would be expected to equal the osmotic pressure of the latex, or in general a force of several atmospheres.

Low humidity, drought, and other factors which set up moisture stress within the plant reduce the volume of flow and thus increase the concentration of the latex obtained. In 1943, for example, the spring and summer seasons were abnormally dry and high temperatures prevailed. During this time latex yields usually were higher on cool, cloudy days than on hot, sunny ones. Low relative humidity was directly correlated with low yields of latex, and winds caused further reductions in the volume of latex. Under these conditions the concentration of solids in the latex increased, probably because of loss of water from the latex vessels through water stress in the whip and the whole plant. Similar observations on latex flow in *Crypto-stegia grandiflora* were made in Haiti by Curtis and Blondeau (5).

<center>DILUTION OF LATEX DURING EXUDATION</center>

When a *Cryptostegia grandiflora* whip is bled, the latex as it exudes becomes progressively more dilute. This response is illustrated in table 1, which is based on the amount of alcohol-coagulable material contained in successive exudations of latex from whips bled October 4, 1943, at Matamoros, Mexico. The latex collected during the first 15 seconds of exudation was four times as concentrated as that collected during the third and fourth minutes. The initial sample was twice as concentrated as the average for the entire bleeding.

TABLE 1.—*Average latex and concentration of coagulate in latex from whips of Cryptostegia grandiflora during exudation from single bleeding cuts, Matamoros, Mexico, Oct. 4, 1943*

[Each value from 50 whips]

Experiment and period after making cut	Latex per whip	Coagulate in latex	Experiment and period after making cut	Latex per whip	Coagulate in latex
Experiment 1:	*Milligrams*	*Percent*	Experiment 2	*Milligrams*	*Percent*
0–15 seconds	44.5	28	0–15 seconds	13 0	32
16–30 seconds	19.7	24	16–30 seconds	4.9	29
31–60 seconds	39 7	16	31–60 seconds	41.2	21
61–120 seconds	80.9	10	61–120 seconds	61.5	11
121–240 seconds	78.2	6	121–240 seconds	57 9	9
Total or average	263.0	14	Total or average	178.5	15

This dilution of latex during exudation from *Cryptostegia grandiflora* is analogous to the dilution in *Hevea*, which was studied extensively by Arisz (2) and Frey-Wyssling (6, 7). The dilution in *C. grandiflora* is, however, even more pronounced than that in *Hevea*; in a typical case described by Frey-Wyssling, the concentration of total solids in *Hevea* dropped from 39.1 to 33.2 percent during the course of the flow. The cause of the dilution was found by Frey-Wyssling to lie in the water relations of the latex vessel. Before bleeding, the vessel is under turgor pressure and in fact is in approximate water balance so that its suction force is small and its turgor pressure equals the osmotic pressure of its cell contents. When the bleeding cut is made, the turgor pressure is reduced to zero at the point

of the cut; therefore, the suction force of the vessel becomes equal to the osmotic pressure of the cell contents. Because of this, water is removed from the parenchymatous cells surrounding the vessels; as a result, with continued exudation the latex becomes progressively more dilute. The concentration of the latex collected in the initial period therefore gives a minimum figure for the concentration of the latex in the intact whip before bleeding.

A similar progressive diminution in latex concentration was found in whips bled daily for several days. Table 2 shows that in the course of five successive daily bleedings the concentration of total solids in the latex dropped in one experiment from approximately 22 percent at the first bleeding to 6.7 percent at the last. Similar behavior was repeatedly noted in other experiments.

TABLE 2.—*Average concentration of total solids in latex from mature whips of Cryptostegia grandiflora without growing tips bled on successive days, Ciudad Victoria, Mexico, Feb. 16–24, 1945*

[Each value from 30 whips]

Bleeding	Interval between bleedings	Total solids in latex from group—					
		1	2	3	4	5	6
	Hours	*Percent*	*Percent*	*Percent*	*Percent*	*Pcecent*	*Percent*
1		21.9	21.8	22.0	21.9	22.3	21.9
2	24	11 9	11.3	11.4	11.1	11.1	11.2
3	24	9.5	10.2	9.7	9.9	9.9	9.7
4	24	8.2	8.7	8.3	8.7	8.6	8.6
5	24				6.7	6.7	6.6

RECONCENTRATION OF TOTAL SOLIDS IN LATEX

Arisz (*2*) and Frey-Wyssling (*6, 7*) found that when the flow of *Hevea* latex ceases, because of the coagulation of the latex at the tapping cut, there is a reconcentration of the total solids in the latex still contained in the vessels. This is apparently due partly to the formation of new rubber in the latex and partly to osmotic removal of water from the diluted latex by the surrounding parenchymatous cells as the turgor of the vessels is reestablished.

The possible existence of a similar reconcentration in *Cryptostegia grandiflora* whips was investigated. At each of four or five daily bleedings half an inch of whip was removed from six comparable groups of whips. As stated previously, by this procedure the concentration of total solids in the latex was reduced from approximately 22 percent at the first bleeding to 6.7 percent at the fifth (table 2). For the next bleeding a 2½-inch piece of whip was removed at periods ranging from 0 to 96 hours after the preceding bleeding. The results were as follows:

Group:	Period after 24-hour bleeding (hours)	Total solids in latex (percent)	Group—Con.	Period after 24-hour bleeding (hours)	Total solids in latex (percent)
1	0	7. 5	4	48	8. 2
2	6	7. 5	5	72	8. 1
3	24	6. 8	6	96	8. 3

Even after 96 hours the concentration of total solids in the latex was far below that obtained at the first bleeding and was scarcely higher

than that obtained immediately after the fifth bleeding (table 2). This experiment offers no evidence of any considerable reconcentration of total solids in latex after bleeding in *C. grandiflora* whips.

In other experiments whips were bled at various periods after a single bleeding. The latex was collected at intervals up to 100 seconds so that the dilution during latex flow could also be studied. During the first bleeding a marked dilution occurred (table 3). When the second bleeding cut was made immediately after the initial one, the first latex exuded was more concentrated than the last obtained from the first cut. The reason for this may be that the latex exuded from the second cut comes partly from regions of the whip which did not supply latex during the initial flow. No reconcentration of the latex total solids was apparent after 10 or 50 minutes; in fact, latex collected 50 minutes after the first bleeding appeared to be even more dilute than that collected immediately after the first cut was made. This probably indicates that the dilution in *Cryptostegia grandiflora* (the passage of water from other tissues into the latex vessels) proceeds to some extent even after latex has ceased exuding. Furthermore, no reconcentration of the latex occurred in periods up to 24 hours after a cut was made (table 3).

TABLE 3.—*Average concentration of total solids in latex from succulent whips of Cryptostegia grandiflora with growing tips bled by making a second cut 1 inch below the initial at various intervals, Llera, Mexico, Apr. 10–12, 1945*

[Each value from 24 whips]

Experiment, date, and period after bleeding	At initial bleeding	Total solids in latex—						
		When a second cut was made after—						
		0 minute	10 minutes	50 minutes	1½ hours	4 hours	18 hours	24 hours
	Per- cent	*Per- cent*	*Per- cent*	*Per- cent*	*Per- cent*	*Per- cent*	*Per- cent*	*Per- cent*
Experiment 1:								
0–10 seconds	28.7	18.6	16.9	14.8				
11–20 seconds	17.5	17.9	16.3	9.3				
21–50 seconds	15.6	16.6	12.4	10.8				
51–100 seconds	13.9	16.1	11.8	7.5				
Experiment 2:								
0–10 seconds		13.2			10.9	10.6	11.9	11.0
11–20 seconds		12.6			9.3	9.9	10.5	10.7
21–50 seconds		9.7			9.1	8.2	8.5	9.8
51–100 seconds		6.8			6.5	7.8	6.1	

The data indicate that under the conditions of these experiments no considerable reconcentration of latex occurs within 10 minutes to 96 hours after bleeding. This is in contrast with *Hevea*, in which Arisz (2) found that a concentration of total solids equal to that of the initial latex after a single bleeding may be reestablished within approximately 12 hours.

LATEX MOVEMENT IN WHIPS AFTER A BLEEDING CUT

To study latex movement in whips after a bleeding cut had been made, latex vessels were internally blocked at various distances below the cut by freezing a short length of the whip with solid carbon

dioxide. Two pieces of solid carbon dioxide each having a volume of several cubic inches were held against the whip so that about a ½-inch length of stem was frozen. The stems were usually frozen solid within 30 seconds, but 60 to 90 seconds was ordinarily allowed to insure complete solidification. The points of freezing were at predetermined distances below the places where the cuts were to be made. The bleeding cuts were in the most apical living internode of mature whips whose terminal internodes had been killed by strong winds. Yield of latex was determined for 60 seconds after a cut had been made. Freezing did not appear to injure adjacent vessels, for when the frozen portion was cut off the remaining stump bled freely. When whips were frozen 40 inches below the apical cut, only a very slight diminution in yield resulted as compared with that from non-frozen whips (table 4). When the distance below the bleeding cut was reduced to 20, 10, or 5 inches, further reductions in yield of latex occurred. From these data it may be calculated that the yield would have been reduced to half that from nonfrozen whips if the frozen block had been 18.5 inches below the bleeding cut. In other words, half of the latex yield from an initial bleeding cut in a whip originated within 18.5 inches of the cut.

TABLE 4.—*Latex flow after whips of Cryptostegia grandiflora had been frozen at various distances below the bleeding cuts made in upper third of the most apical living internode, Ciudad Victoria, Mexico, Feb. 17, 1945*

Distance between apical cut and frozen area	Plants treated	Latex flow per whip during first minute	
		At apical cut	Half inch below frozen area
	Number	*Drops*	*Drops*
Control (nonfrozen)	12	12.7	
40 inches	5	11.5	11.0
20 inches	6	6.8	9.5
10 inches	6	3.1	11.0
5 inches	7	1.1	11.6

In a second similar experiment the latex was collected at shorter time intervals than in the first to determine the length of whip contributing to the yield of latex during different periods of flow (table 5). At the various distances below the cut the effect of each block increased with time. Thus, although a block 20 inches below the cut

TABLE 5.—*Latex flow after whips of Cryptostegia grandiflora had been frozen at various distances below the bleeding cuts made in upper third of the most apical living internode, Ciudad Victoria, Mexico, Feb. 23, 1945*

Distance between apical cut and frozen area	Plants treated	Latex flow per whip during seconds indicated				
		0-10	11-20	21-40	41-60	0-60
	Number	*Drops*	*Drops*	*Drops*	*Drops*	*Drops*
Control (nonfrozen)	12	3.8	2.2	2.2	1.3	9.5
40 inches	12	3.6	2.1	2.1	1.2	9.0
20 inches	12	3.3	1.7	.8	.3	6.1
10 inches	12	1.6	.6	.3	.2	2.7
5 inches	12	.2	.3	.2	.1	.8

did not reduce latex flow during the first 10 seconds after the cut was made, thereafter it caused a marked reduction. As in the first experiment, the distances between the frozen block and the cut necessary for reduction of the latex flow to half that from the nonfrozen whips were calculated. The values obtained were as follows:

Period after making the bleeding cut:

	Distance necessary (inches)
0–10 seconds	11. 6
11–20 seconds	14. 2
21–40 seconds	24. 2
41–60 seconds	26. 8
0–60 seconds	16. 2

These data indicate that the length of whip contributing to the yield of latex more than doubled during the 60 seconds of flow. Similar results in *Hevea* were obtained by Arisz (*2*) and Frey-Wyssling (*6, 7*). The results may indicate that the lowering of the hydrostatic pressure in the vessels as a result of the incision is slowly extended along the whip.

In a third experiment the length of whip involved in latex flow was studied on a day when air temperature was low and there was a drying wind. Under these conditions the distance between block and bleeding cut necessary for reduction of the flow to half that of nonfrozen plants was only 11 inches, or considerably shorter than that found in the first two experiments. Under conditions of moisture stress latex flow is apparently confined to a shorter distance than under more favorable conditions.

These data indicate that after an initial whip bleeding cut 90 percent or more of the latex yield originates within 40 inches of the cut and that 50 percent originates within 10 to 20 inches, these distances varying according to prevailing environmental conditions. These conclusions were substantiated in a fourth experiment in which whips were subjected to two simultaneous bleeding cuts, an apical and a basal cut (table 6). This experiment, conducted on the same day as that reported in table 4, indicated, as in that experiment, that the yield of latex was reduced by half when each cut drew on only 18.5 inches of whip (p. 110).

These conclusions in respect to the length of whip involved in latex flow do not give exact information as to the distance that the latex actually exuded may have traveled within the whip. At least

TABLE 6.—*Latex flow from an apical cut in upper third of the most apical living internode of whips of Cryptostegia grandiflora made simultaneously with a basal cut, Ciudad Victoria, Mexico, Feb. 17, 1945*

Distance between apical and basal cuts	Plants tested	Latex flow during first minute	
		At apical cut	At basal cut
	Number	*Drops*	*Drops*
Control [1]	2	13. 0	
80 inches	8	11. 9	10. 1
40 inches	6	7. 3	8. 0
20 inches	5	2. 6	2. 8
10 inches	2	1. 0	. 5

[1] See also the control in table 4 as the experiments were conducted on the same day.

two complications arise in determining these distances. The first is the dilution of exuding latex. Part of the latex exuded was not present in the latex vessels before the bleeding but entered the vessels as water after the bleeding cut was made. The volume of this water may be so great as to equal that of the original latex (table 1). Furthermore, water uptake at any given point in the vessel depends on the extent to which turgor pressure has been decreased at that point as a result of the bleeding cut. The second complication is based on the relation between distance from the incision and the turgor pressure of the latex vessel. The nature of this relation is not only unknown, but it also changes with time. It is apparent that until data are available on these points the actual length of whip from which the exuded latex originates will remain a matter of conjecture.

LATEX YIELD AND FACTORS AFFECTING IT

WHIP-BLEEDING YIELD ACCORDING TO INTERNODE

When whips are bled for the first time, the yield of latex varies according to the position of the internode which is cut. Therefore, the internodes of the whips to be cut were numbered basipetally, starting with the first internode that was more than an inch long. In several experiments the highest yielding internode was found to be the seventh from the topmost (table 7). In all cases yield was

TABLE 7.—*Latex and coagulate per whip of 1-year-old Cryptostegia grandiflora plants after 3 successive bleedings of various internodes followed in each case by an additional bleeding in the next internode, Matamoros, Mexico, 1943*

[Each value from 50 whips]

LATEX

Internode bled	Sept. 17	Sept. 18	Sept. 19	Sept. 20	Total for Sept. 17–19
	Grams	*Gram*	*Gram*	*Gram*	*Grams*
3	0 399	0. 206	0. 333	0. 520	0. 938
5	. 592	. 321	. 388	. 579	1 301
7	. 828	. 437	. 455	. 687	1. 720
9	. 988	. 418	. 460	. 672	1. 866
12	1. 190	. 427	. 362	. 562	1. 979
15	. 923	. 169	. 101	. 162	1 193
18	. 817	. 046	. 022	. 058	. 885

TOTAL COAGULATE

3	0. 064	0. 023	0. 039	0. 054	0. 126
5	. 092	. 035	. 045	. 075	. 172
7	. 143	. 048	. 050	. 089	. 241
9	. 135	. 042	. 045	. 091	. 222
12	. 144	. 042	. 030	. 046	. 216
15	. 127	. 020	. 007	022	. 154
18	. 123	. 007	. 002	. 004	. 132

COAGULATE IN LATEX

	Percent	*Percent*	*Percent*	*Percent*	
3	15. 7	10. 3	10. 3	9. 1	
5	14. 8	9. 4	9. 0	8. 8	
7	16. 7	8. 9	7. 3	7. 6	
9	12. 9	8. 0	6. 7	7. 8	
12	10. 8	7. 4	6. 6	6. 5	
15	11. 7	8. 2	6. 4	7. 4	
18	11. 5	12. 0	7. 4	1. 0	

increased when after three bleedings within an internode the fourth bleeding cut was made in the next internode.   This increase resulted mainly from an increase in volume of latex and not from an increase in the concentration of the coagulate in the latex.   Apparently the nodes tend to restrict the flow of the latex.   The percentage of coagulate in latex was always higher from the first bleeding in an internode than from subsequent bleeding cuts in the same internode.   A comparison of decrease in yield of latex coagulate during three successive bleedings at 24-hour intervals within each of several internodes showed that the third internode decreased 39 percent, the fifth 51 percent, the seventh 65 percent, the ninth 67 percent, the twelfth 79 percent, the fifteenth 94 percent, and the eighteenth 98 percent. From the data it is apparent that the initial bleeding of a series should be made from the third to the sixth internodes, inclusive.

YIELD OF LATEX FROM VARIOUS BLEEDING SCHEDULES

Numerous experiments were carried out to determine the yield of latex obtainable from whips under various bleeding schedules. Bleedings were started at different times during the development of the whip, and the schedules were varied as to length of stem removed at each bleeding and interval between bleedings.   Each bleeding schedule was usually carried out on samples of 50 to 125 whips.   The data from a wide variety of such experiments are summarized in table 8.

The highest yields of alcohol coagulate, on a per whip basis, were obtained when bleedings were started in the third to sixth internode and 1 to 1½ inches of whip was removed at each subsequent cutting with an interval of 48 or 72 hours between bleedings.   Almost as large yields per whip were obtained with four bleedings per internode followed by a 4- to 7-day interval between bleedings in each internode. Approximately 2 gm. of coagulate per whip was obtained by exhaustive bleeding of average-length whips on 1½- to 2-year-old plants under the conditions of these experiments.

It is of economic interest to consider the coagulate yield per bleeding.   The highest yield calculated on this basis was obtained by a schedule of bleeding only once per internode with a 3- to 5-day lapse between cuttings.   This schedule yielded a third to a half the amount obtained by exhaustive bleedings but necessitated only a fourth to a third as many bleedings.   Estimates of rubber production by whip bleeding indicate that since approximately 40,000 whips per acre are produced annually under the conditions of these plantings, an annual potential yield of about 175 pounds of 85- to 90-percent rubber per acre would be indicated.   It seems unlikely that more than two-thirds of the experimental yield could be obtained in commercial practice, so that a yield of about 120 pounds of 85- to 90-percent rubber per acre per year from 2-year-old plants of *Cryptostegia grandiflora* might be expected from a conservative whip-bleeding program.

TABLE 8.—*Average yield of latex coagulate per bleeding of 5- to 7-inch internodes of Cryptostegia grandiflora on different bleeding schedules*

[Each value from 50 whips or more]

Period between successive bleedings of a single internode	Period between last bleeding of one internode and first bleeding of next	Whip length cut off	Internode bled first [1]	Total bleedings	Average yield per bleeding
	Hours	*Inches*		*Number*	*Milligrams*
½ hour		0.5	3	4	28
1 hour		.5	3	4	34
4 hours	20	.5	3	26	27
Do.[2]	120	.5	3	19	36
8 hours	96	[3]2.3	---------	12	19
24 hours		5	3	26	31
Do.[2]	120	.5	3	14	51
24 hours		1 0	7	15	42
Do		[3]7.0	---------	14	50
Do		[3]3.5	---------	25	48
Do		[3]1 8	---------	25	46
Do	24	[3]7.0	---------	12	19
Do	96	[3]2.3	---------	12	22
Do	96	.5	---------	12	21
Do	168	[3]2.3	---------	9	23
Do	240	[3]2 3	---------	9	26
48 hours		1.0	3	50	50
Do		1.0	6	47	49
Do		1.5	3	48	56
Do		1.5	6	45	57
Do		[3]7.0	---------	14	67
Do		[3]3.5	---------	25	62
Do		[3]1 8	---------	25	55
Do	48	.5	3	11	46
Do	72	.5	3	11	51
Do	120	.5	3	11	48
Do	168	.5	3	9	49
Do	240	.5	3	9	46
Do	120	1.0	3	12	64
Do	48	---------	---------	12	21
72 hours		---------	3	10	33
Do		[3]7.0	---------	14	88
Do		[3]3.5	---------	25	65
Do		[3]1.8	---------	25	57
96 hours		1.0	3	8	39
120 hours		[3]7 0	---------	7	93

[1] Numbered basipetally.
[2] Only 4 bleedings per internode.
[3] Length cut off based on an average internode length of 7 inches.

EFFECT OF PREVIOUS BLEEDING ON LATEX YIELD

From the economic point of view it is of interest to know the influence of continued bleeding on yield of rubber. For this purpose an experiment was carried out to determine the effect of repeated bleedings on the amount of alcohol-coagulable material of latex. In this experiment one group of whips was bled four times per internode at 24-hour intervals. At each time a new internode was tapped, a fresh group of previously nontapped whips was bled in the corresponding internode for comparison. The data in table 9 show that the yield of coagulate decreased greatly with repeated tapping. At the time of the twenty-fifth bleeding (the first bleeding in the twelfth internode), for example, whips bled four times per internode at 24-hour intervals yielded a total coagulate of 0.041 gm. per whip. This low yield was due both to a low coagulate content of the latex (9 percent) and to a low yield of latex. The comparable previously nonbled whips yielded latex having 17 percent of alcohol coagulate and a total coagulate of 0.187 gm. per whip.

TABLE 9.—*Yields of total coagulate from repeatedly bled and once-bled 7- to 9-foot whips on cultivated, 15-month-old plants of Cryptostegia grandiflora*

[Bleedings every 24 hours, 4 per internode; each value from 50 whips]

| Internode bled [1] | Single bleeding per whip | Successive bleedings of the same whip | | |
		First bleeding in inter-node	Total per inter-node	Cumula-tive yield
	Gram	*Gram*	*Gram*	*Grams*
6	0.110	0.133	0.323	0.323
7	.112	.059	.180	.503
8	.112	.058	.229	.732
9	.114	.055	164	.896
10	.146	.041	.096	.992
11	.169	.048	.106	1.098
12	.187	.041		

[1] Numbered basipetally.

It would appear that the observed increase in latex yield and rubber content from whips bled at intervals of 72 hours as compared with those bled at shorter intervals resulted, in part at least, from the drawing of latex from farther down in the whip. These data agree with those reported under latex-movement studies.

COMPARISON OF LATEX OBTAINED BY BLEEDING WITH THE TOTAL IN THE WHIP

It would be of interest to know whether rubber is formed in the whip during the course of bleeding or whether exhaustive bleeding merely drains the whip, and perhaps even the plant, of rubber initially present. Data presented in the foregoing sections indicate that no detectable production of rubber takes place in the latex vessels during the usual bleeding intervals. Nevertheless, 539 mg. of rubber hydrocarbon was obtained per whip from 18 bleedings, whereas that originally present as shown by benzene extraction of comparable nonbled whips was only 240 mg. This difference would be further increased if the maximum number of bleedings possible, about 50, had been made.

The following experiment also indicates that the bleeding process itself may result in the development of a small amount of rubber not detectable by present methods of analysis. Sixteen groups of thirteen randomized whips each were selected and tagged on the plants. These whips were 2 to 3 feet long. The terminal 15 inches was cut off from the plants in six groups, and the cut surface of the excised part was immediately plunged into alcohol to prevent latex flow. A second lot of six groups was similarly treated except that they were bled after being cut from the plant in order to obtain all the latex possible. A third lot of four groups was bled just as the second lot was. The whips and latex of all lots were then dried and subjected to rubber analysis by the benzene-extraction method. In the second lot the dried latex rubber was analyzed separately from the whip, whereas in the third lot the latex rubber was blended with the whip for analysis as a check on recovery of the rubber.

The bled whips yielded significantly more total rubber than the nonbled controls and the yield increase approximated the amount of rubber obtained in the latex (table 10). This result, which was also obtained in two other experiments, might be taken to indicate that the extraction of rubber from the whip material was incomplete or that drying the whip before extraction destroyed the rubber. The first possibility seems remote, since exhaustive extraction of the woody whip material did not alter the result and also 100-percent rubber recovery was obtained in the third lot when rubber was added to the whip material before analysis. It appears that under some circumstances and with young whips, which were used in the present experiments, either the act of bleeding results in the formation of rubber or drying the whips destroys the rubber. This phenomenon further complicates studies on rubber formation in whips.

TABLE 10.—*Yield per whip of benzene-extractable material from cut-off terminal 15 inches of young 2- to 3-foot whips of Cryptostegia grandiflora bled and not bled after cutting, 1 to 3:30 p. m., Dec. 11, 1944*

[Latex and whips air-dried at 60° C.; each random sample average of 13 whips]

		Yield from bled whips when—					
Yield from nonbled whips			Latex and whip material separated			Latex and whip material together	
Sample No.	Whip material	Sample No.	Whip material	Latex	Total	Sample No.	Latex and whip material
	Milligrams		*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*
447	20.8	453	13.8	8.0	21 8	466	23.1
448	23.0	454	16.4	7.0	23.4	467	22.5
449	19.0	455	15.6	8.7	24.3	468	22.4
450	13.2	456	15 2	6.4	21.6	469	29.2
451	15 5	457	19 2	7 6	26 8		
452	17.3	458	20.2	7.6	27.8		
Average	18.1		16 7	7.6	24.3		24.3
Increase over nonbled whips					[1] 6.2		[2] 6.2

[1] Statistically significant at 1-percent level.
[2] Statistically significant between 2- and 3-percent levels.

RELATION OF LEAF RUBBER TO WHIP LATEX RUBBER

It has been mentioned previously that leaves of *Cryptostegia* contain both latex and cellular rubber. To determine whether there is a correlation between the rubber in the leaves and that in the latex, latex-rubber yield and leaf-rubber content were determined on a series of individual plants. Single whips on each of 10 individual plants were given 15 consecutive bleedings at 24-hour intervals. The latex was dried at 60° C. The yield from all the 15 bleedings was pooled for each whip and analyzed. Leaf samples from the same plants were similarly dried at 60° and analyzed. The rubber in the latex varied from 52.1 to 67.6 percent and that in the leaves from 1.39 to 3.02 percent. No correlation was found between the leaf rubber or resins and the whip latex-rubber content or yield. On the basis of present information, the absence of such correlations would necessitate latex

analyses and yield determinations on individual plants in connection with any breeding program for the development of high-yielding strains of plants with high-quality latex.

LATEX COMPOSITION AND FACTORS AFFECTING IT

CHEMICAL COMPOSITION OF LATEX

The latex of *Cryptostegia grandiflora* contains 5 to 25 percent of total solids, depending on the plant and on the circumstances under which the latex is collected. The solids are partly in the form of latex particles which are held in suspension in an aqueous serum. The latex particles were studied with the aid of the electron microscope by Hendricks, Wildman, and McMurdie (*9*), who showed that they vary in diameter from a few tenths of a micron to possibly 1μ and are approximately spherical. Vigorous mechanical agitation of the latex results in coagulation of the particles, which may then be completely separated from the serum by repeated washing.

The whole latex of a composite sample of *Cryptostegia grandiflora* collected at Llera, Mexico, on March 14, 1945, was found on fractionation to consist of 14 percent of mechanical coagulate and 86 percent of serum. The coagulate consisted of a small amount of protein, a substantial amount of acetone-soluble material (resins), and a very large amount of acetone-insoluble, benzene-soluble material, which is assumed to be rubber (table 11). The acetone-soluble, water-insoluble fraction would contain the triterpene ester studied by Hendricks and Wildman (*8*), who indicated that this compound may constitute as much as 90 percent of the fraction in latex of *C. madagascariensis* but that only a small amount is contained in the corresponding fraction of latex of *C. grandiflora*. Whether the resins are contained in the same latex particles as the rubber or in separate particles cannot be stated with certainty. The protein, however, probably represents material held on the surface of the particles, and as such it is to be regarded as a particle constituent. Tristram (*17*) indicated that in *Hevea* latex these particle-bound proteins are similar to the proteins of the latex serum, at least in amino acid composition.

TABLE 11.—*Latex constituents of a composite sample of Cryptostegia grandiflora collected at Llera, Mexico, Mar. 14, 1945*

Material	Solids in latex	Mechanical coagulate	Solids in serum
	Percent	*Percent*	*Percent*
Rubber (benzene-soluble)	57.1	[1] 84.4	-----------
Resins associated with rubber	7.2	10.7	-----------
Protein associated with rubber	2.1	[2] 3.1	-----------
Acetone-soluble material:			
Citric acid	.3	-----------	0.8
Malic acid	.1	-----------	.3
Unknown serum resins	2.3	-----------	6.9
Phenol [3]	19.5	-----------	59.0
Potassium chloride	4.6	-----------	[4] 14.0
Amino acids	3.7	-----------	[5] 11.2
Protein of serum	3.0	-----------	[6] 9.1
Total	99.9	98.2	101.3

[1] After acetone extraction.
[2] Percent of N × 6.25.
[3] Derived from data of Stewart and Hummer (*15*).
[4] Based on analysis of comparable samples.
[5] α-Amino N × 7.
[6] Insoluble N × 6.25.

The serum of latex of *Cryptostegia grandiflora* may contain various amounts of total solids, which can be fully accounted for in terms of relatively well-known components. The representative latex of table 11 consisted of 7.9 percent of total solids and 92.1 percent of water. Of the solid material in the serum, 8 percent was acetone-soluble; this included small amounts of citric and malic acids determined by the methods of Pucher, Wakeman, and Vickery (*13*). The rest of the acetone-soluble material, however, was of unknown nature. The principal constituent of the serum solids was the substance described by Stewart and Hummer (*15*), an acetone-insoluble, ethyl-alcohol-soluble phenolic substance. This substance is of particular interest because large amounts of it were found in low-rubber latices and small amounts in high-rubber latices. That the substance is a phenol is indicated by its qualitative reactions, but its structure is not known. The serum further contained amino acids, potassium chloride, and protein. The amino acid content of *C. grandiflora* latex has a counterpart in the amino acids found in *Hevea* latex by Whitby and Greenberg (*18*) and Altman (*1*). None of the amino acids of *C. grandiflora* latex has as yet been isolated and identified, and their existence is necessarily based on the α-amino nitrogen content of the latex.

It was found that the proteins of *Cryptostegia grandiflora* latex contain a peroxidase. Freshly prepared serum was treated with an equal volume of saturated ammonium sulfate. The resulting precipitate was taken up in 0.05 M phosphate buffer, pH 5.5, the pH of the serum. In the presence of hydrogen peroxide this protein preparation was able to carry out rapid oxidation of *p*-cresol, guaiacol, β-naphthol, pyrogallol, *p*-phenylenediamine, and the latex phenolic substance of Stewart and Hummer (*15*). The reaction appears to be enzymatic, as indicated by inactivation of the peroxidase activity by prolonged heating at 100° C. It is of interest that the phenol, which is a normal constituent of the serum, is oxidized rapidly to an orange-colored product. In the freshly collected latex immediate and enzymatic darkening of the latex also takes place on the addition of hydrogen peroxide. This indicates that a significant amount of hydrogen peroxide does not accumulate in latex in the latex vessels.

Catalase activity was found to be very slight in latex of *Cryptostegia grandiflora*, but high in latex of *C. madagascariensis* and in that of the hybrid. Polyphenoloxidase and tyrosinase were lacking in the serum of *C. grandiflora*. Other enzymes were not investigated.

That enzymes occur among the serum proteins of other latices was indicated by Spence (*14*), who found peroxidase in *Hevea* latex. The latex of *Ficus* sp. is particularly rich in peroxidase (*16*), and proteolytic enzymes accumulate in other latices such as those of *Carica papaya* L. and *Asclepias* sp.

LATEX COMPOSITION AS AFFECTED BY BLEEDING

Analyses of latex total solids indicated that the ratio of rubber hydrocarbon to insolubles decreases in bleedings made within half an hour after an initial bleeding (table 12). This trend continues for two additional bleedings at ½-hour intervals. The composition of latex from a second bleeding 1 hour after the initial bleeding was practically the same as that from the third bleeding at ½-hour intervals. Appar-

ently latex composition is in part a function of lapsed time after the first bleeding as well as of the total number of bleedings.

TABLE 12.—*Average composition of pooled latex total solids in duplicate analyses of completely randomized samples from whips of Cryptostegia grandiflora bled successively at different intervals*

[Each value from 50 whips]

Component and period after first bleeding	Portion of total solids with indicated bleeding interval (hours)			
	1½	1	4	48
Rubber hydrocarbon:	*Percent*	*Percent*	*Percent*	*Percent*
0 hour	61.6	62 8	61 9	61.3
½ hour	60 4			
1 hour	57.8	58 7		
1½ hours	54.1			
2 hours		54.5		
4 hours			54.1	
32 days				54.8
Insolubles.				
0 hour	21 7	21 1	23 4	26 1
½ hour	22 7			
1 hour	26 2	26.4		
1½ hours	30.8			
2 hours		30.5		
4 hours			31.0	
32 days				29.2
Resins:				
0 hour	16 7	16.1	14.7	12.6
½ hour	16 9			
1 hour	16 9	14.9		
1½ hours	15.1			
2 hours		15.0		
4 hours			14.9	
32 days				16.0

Latex was also obtained by a single initial bleeding from the upper, middle, and lower portions of whips. The yield from 50 samples of each portion was composited, dried at 60° C., and analyzed. There was 46.5 percent of rubber in the latex from the upper portion, 47.1 percent in that from the middle, and 52.6 percent in that from the lower. These data agree with the general observation for rubber-producing plants that old tissues usually have higher percentages of rubber than young ones.

Analyses of representative samples of air-dried latex collected after various consecutive bleedings in the course of different bleeding schedules are shown in table 13.

Latex obtained from the first bleeding of a whip always had a higher content of rubber hydrocarbon and a lower one of insolubles than that from subsequent bleedings. The latex total solids from the first bleeding usually contained 60 to 65 percent of rubber hydrocarbon and 21 to 24 percent of insolubles. Except in one of the schedules latex from the final bleeding was always lower in rubber hydrocarbon than from any of the intermediate bleedings. Rubber hydrocarbon content of the latex from the intermediate bleedings changed erratically. It was not possible to correlate these changes with any known factors such as weather, internode, or bleeding schedule. These data indicate that during the course of bleeding schedules through as much as 100 days enough synthesis of rubber hydrocarbon did not take place in the whip to equal that in the latex at the first bleeding.

TABLE 13.—*Composition of pooled representative samples of air-dried latex, from consecutive bleedings of 9- to 11-foot whips of Cryptostegia grandiflora, Llera, Mexico, first bleedings, June 22–25, 1944*

[Each value from 50 whips]

Sample	Bleeding schedule — Whip length cut off	Bleeding interval	Inter-node bled first	Bleeding	Portion of latex — Rubber hydrocarbon	In-solubles
	Inches	Hours			Percent	Percent
1	1	48	3	1	62.5	21.1
				15	52.8	31.3
				18	55.0	29.8
				19	54.4	26.2
				23	57.7	24.0
				50	43.9	28.6
2	1	72	3	10	54.8	30.3
3	1	96	3	8	56.2	30.3
4	1½	48	3	15	51.2	34.0
5	1	48	6	15	54.0	30.6
				18	55.4	30.8
				19	50.2	32.8
				23	49.6	32.1
6	1	¹ 48	3	4	56.2	28.6
				12	52.9	33.3
7	½	24	3	26	52.9	33.3
8	½	(²)	--------	1	61.9	23.4
				2	54.1	31.0
				15	44.4	42.6
				16	45.9	40.8
				27	47.4	40.7
				28	48.0	40.2
				37	47.0	37.8
				38	45.2	38.6
				39	51.3	35.7
				40	47.2	38.0
				45	56.0	30.0
				46	51.2	36.3
9	½	(¹²)	3	5	51.2	35.6
				6	51.7	36.7
				9	46.5	41.2
				10	49.0	39.1
				11	57.5	31.6
				12	57.3	28.2
				13	50.9	34.0
				14	49.4	36.2
				15, 16	56.4	30.3
				17	50.1	32.3
10	1½	(¹³)	3	13	54.8	30.5
				14	53.5	32.9
11	1½	40	6	21	57.5	25.9
12	1½	48	3	21	61.5	19.1
				48	45.7	31.2

¹ Within each internode 4 bleedings were made as indicated, but there was a 5-day interval before bleeding in the next internode.   ² Twice daily.   ³ Daily.

EFFECT OF TIME OF DAY ON LATEX COMPOSITION

Analyses of latex total solids from bleedings at either 6:15 a. m. or 3 p. m. from two groups of whips from October 23 to 28, 1944, are given in table 14. After the first three bleedings the schedules were reversed so that the group originally bled at 3 p. m. was bled at 6:15 a. m. and vice versa. On a percentage basis the rubber hydrocarbon was higher in the morning latex than in the afternoon, the difference being statistically significant at the 2- to 3-percent level. Insolubles were higher in the afternoon latex than in the morning, the difference being significant at the 1- to 2-percent level. In these computations the results for October 26 were omitted because the time intervals between successive bleedings of the morning and afternoon groups

differed owing to the previously mentioned change in the bleeding schedule. None of the differences in latex composition could be ascribed to sunlight intensities or other weather conditions.

TABLE 14.—*Average composition of latex total solids in duplicate analyses of latex from completely randomized 7- to 9-foot whips of Cryptostegia grandiflora bled at different times of day, Oct. 23–28, 1944*

[Each value from 50 whips]

Internode and date	Temperature range during preceding night	Relative humidity at 3 p. m.	Portion of latex total solids			
			Insolubles		Rubber hydrocarbon	
			6·15 a.m.	3 p. m.	6:15 a.m.	3 p. m.
	° F.	Percent	Percent	Percent	Percent	Percent
Internode 5·						
Oct. 23	80–58	63	22	26	61	58
Oct. 24	81–60	56	27	30	58	57
Oct 25	80–55	58	37	42	50	45
Internode 6·						
Oct. 26	79–56	80	¹ 44	² 22	¹ 43	² 62
Oct. 27	74–62	76	40	43	45	42
Oct 28	77–61	84	40	50	43	36

¹ Whips bled Oct. 25, 1944, at 3 p. m.
² Whips bled Oct. 25, 1944, at 6 : 15 a. m.

The evidence from two earlier experiments, together with the data in table 14, indicates that latex obtained after the night, or dark, period is higher in percentage of rubber hydrocarbon and lower in insolubles than that obtained after the day, or light, period. Such results, however, do not necessarily imply that insolubles are synthesized during the day and rubber hydrocarbon during the night, since they are on a percentage rather than actual-weight basis for these components.

LATEX YIELD AND COMPOSITION FROM FOLIATED AND DEFOLIATED PLANTS

Two groups of twenty 10-month-old plants were selected for uniformity of size. One group was then completely defoliated by hand. Three days later comparable-sized whips in both groups were bled for eight daily bleedings with four bleedings per internode. The latex from the first four bleedings was alcohol-coagulated, whereas that from the second four was air-dried.

The foliated plants consistently yielded more coagulate than the defoliated ones (table 15), the difference amounting to about 12 percent for the first bleeding but increasing to about 40 percent for all of the remaining bleedings. Yield of latex was greater from the defoliated plants for the first two bleedings; thereafter the foliated plants yielded more latex, which had a higher percentage of rubber. Such results may indicate that the leaves are essential for production of coagulate in latex, as well as for maintaining the flow of latex during a number of bleedings. The greater flow from the defoliated plants for the first two bleedings was probably a direct effect of the removal of the leaves. In this respect, the artificially defoliated plants were similar to naturally defoliated ones studied in western Mexico.

TABLE 15.—*Coagulate per whip from consecutive daily bleedings of foliated and defoliated plants of Cryptostegia grandiflora*

[Each value from 20 plants]

Condition of plants and bleeding No.	Kind of latex	Latex	Latex coagulate	Stem coagulate	Total coagulate	Coagulate in latex
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*
Foliated.						
1	Alcohol-coagulated	213	34.2	24 3	58.5	15.9
2	do	168	7 5	5.2	12.7	4.5
3	do	162	10 5	2.0	12.5	6.5
4	do	175	5 0	1 7	6.7	2 8
5	Air-dried	182	11.7	3.5	15 2	6.4
6	do	152	10.7	1 0	11.7	7.0
7	do	138	8.7	.8	9.5	6.3
8	do	133	8.7	.3	9.0	6.5
Defoliated:						
1	Alcohol-coagulated	247	35.2	16 3	51.5	14.2
2	do	176	6.7	1 0	7.7	3.9
3	do	132	7.5	.7	8 2	5.7
4	do	127	3.5	.4	3.9	2.8
5	Air-dried	167	7.0	2 7	9.7	4.2
6	do	132	8.2	.6	8 8	6.2
7	do	126	6 5	.5	7.0	5.2
8	do	128	8.0	.2	8.2	6.2

To obtain additional information on the effect of leaves on latex the defoliated plants studied were divided into four different categories of foliation, and a group of the original nondefoliated plants were retained as controls. A summary of the results from periodic bleedings of these groups is shown in table 16.

TABLE 16.—*Latex total solids per whip from plants of Cryptostegia grandiflora under various conditions of foliation, beginning with an original defoliation on January 27, 1944*

[Each value from 5 plants bled in intact internodes]

Condition of plant	Yield on bleeding date indicated					Total yield
	Feb. 24	Mar. 3 [1]	Mar. 15	Mar. 28		
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*	*Milligrams*
Never defoliated (control)	32	42	13	14	8.8	101
With regained foliage	18	41	13	19	12.2	91
Continuously defoliated	22	32	20	9	5.1	83
With defoliated whip but regained foliage on rest of plant	20	37	17	11	7.6	85
Continuously defoliated except for regained foliage on whip	24	41	20	17	11.7	102

[1] Total yield from 3 bleedings at 24-hour intervals in same internode.

It was found that the lower yielding whips were those on the plants which had been continuously defoliated or those whips that were the only parts continuously defoliated. The whips on plants allowed to grow new leaves after the initial defoliation and those on plants on which only the whip was allowed to grow new leaves yielded more nearly the same amount of coagulate as whips on plants which had never been defoliated. Similarly, the whips yielding latex containing lower percentages of total solids were those on plants on which the entire plant or the whip alone had been continuously defoliated. The evidence indicates that leaves are necessary to maintain the

total solids in latex and suggests in addition that the only leaves directly involved in whip bleeding are those on the whip being bled. At the time of year when this experiment was carried out (February and March) there were no apparent effects of defoliation on the relative amounts of rubber hydrocarbon, resins, and insolubles in the latex.

COMPOSITION OF LATEX FROM ROOTS, SEED, AND YOUNG SEEDLINGS

Latex collected from the taproots of over fifty 1-year-old plants of *Cryptostegia grandiflora* contained 30.7 percent of rubber hydrocarbon, 31.7 percent of insolubles, and 37.6 percent of resins. In most cases, the exudation was so slight that scraping the cut surfaces with a knife was necessary in order to collect sufficient latex for analysis. Judging from its composition latex from roots may have a fundamentally different physiology from that of above-ground parts of the plant, since the percentages of rubber hydrocarbon and insolubles in the root latex do not fall along the established regression line for these components (*15*).

To obtain sufficient seedling latex for analysis between 500 and 700 seedlings were grown on paper towels kept moist with a complete nutrient solution. When the seedlings were 3 weeks old and about 2 inches high their cotyledonary leaves were cut off and the small droplet of latex exuding from the stump was collected on a glass slide and dried at 60° C. The yield of latex total solids from all of the seedlings amounted to only 17.9 mg. By a bromination analysis this was found to contain 3.4 percent of rubber hydrocarbon and 42.5 percent of insolubles. Like those of the root latex, these percentages do not agree with the correlation between rubber hydrocarbon and insolubles found in latex from older plants. These data indicate that rubber hydrocarbon occurs in very young seedlings even before they have developed their true foliage leaves and so open the possibility of studying rubber formation in seedlings.

It was not possible to detect rubber hydrocarbon in ungerminated seeds. Approximately 200 gm. of seed was extracted with benzene, but bromination of the extract failed to yield any rubber tetrabromide, which it is expected it would have yielded had the extract contained rubber hydrocarbon.

COMPOSITION OF LATEX FROM INTERSPECIFIC RECIPROCAL GRAFTS

Analyses were made of latex from various combinations of bud and cleft grafts between *Cryptostegia grandiflora, C. madagascariensis,* and the F_1 hybrid. These grafts were made between October 15 and November 15, 1943, at Ciudad Victoria, Mexico. Latex was obtained approximately a year later by bleeding the whips or in the absence of whips by bleeding branches less than 5 mm. in diameter. When shoots (suckers) from the stock also had been produced, they were bled separately from the scion. On some plants 2 different scions had been grafted to the same stock, 1 of the scions being of the same species as the stock. The various combinations of grafts and the analytical results of 15 of the 31 grafts tested are presented in table 17.

TABLE 17.—Composition of total solids from latex obtained by whip or stem bleeding of various reciprocal graft combinations between Cryptostegia grandiflora, C. madagascariensis, and the F₁ hybrid, Ciudad Victoria, Mexico

Plant	Species of stock	Species of scion	Growth of stock bled	Growth of graft bled	Rubber hydrocarbon (Percent)	Insolubles (Percent)	Undetermined (Percent)
15	grandiflora	madagascariensis		Whiplike stem	1.7	40.7	57.6
14	do	do	Sucker whip	Foliage stems	2.0	35.9	62.1
					53.8	30.7	16.5
16	do	do	Sucker whip	Whiplike stem	1.7	41.7	56.6
					56.0	28.6	15.4
28	madagascariensis	grandiflora		Whips	53.5	31.2	15.3
25	do	do	Foliage stem of sucker	do	45.9	38.9	15.2
					1.5	42.5	56.0
27	do	do	Young foliage stem of sucker	Whip	49.8	34.5	15.7
					0.7	38.6	60.7
17	grandiflora	Hybrid	Sucker whip	Foliage stem	28.5	38.1	35.4
					55.0	29.1	15.9
20	do	do	Foliage stem of sucker	Foliage stem	27.9	36.8	35.3
					55.6	29.0	15.4
11	do	do	Whip	Foliage stems	28.0	33.7	38.7
					54.6	28.4	16.9
24	madagascariensis	do	Sucker whip	Foliage stem	45.6	31.3	16.4
					51.8	29.3	16.9
9	Hybrid	grandiflora	Foliage stem of sucker	Whip	38.3	30.7	34.4
					51.1	29.3	18.2
22	do	do	Sucker whip	Whip	36.0	35.0	29.0
					0.5	44.0	54.5
31	madagascariensis	madagascariensis	Foliage stem of sucker	Foliage stem	35.3	26.3	38.4
					1.4	40.7	57.9
32	do	do	Sucker whip	Foliage stem	38.0	27.2	34.8
					1.3	43.7	55.0
33	do	do	Sucker whip	Foliage stem	36.9	25.1	38.0

The data indicate that rootstock has no determining influence on the percentage of rubber hydrocarbon and insolubles in the latex from the scion. Likewise, there was no evidence that the scion influenced the percentages of these substances in latex from the stock. It was not possible to determine whether the stock affected the yield from the scion because of a lack of whips on the scions comparable with those on nongrafted plants.

It appears from these results that the role of the roots in the formation of rubber or of insolubles in the latex is an indirect one at best. This directs attention to the role of the above-ground parts of the plant in the synthesis of these two fractions. The possibility of grafting scions of high yield but low quality upon high-quality stocks to improve the quality of the latex from the scion does not seem promising.

ATTEMPTS AT RUBBER FORMATION IN VITRO

To determine whether latex serum from *Cryptostegia grandiflora* latex prepared as described might contain enzymes or substances capable of bringing about rubber formation in vitro at the expense of substances already in the latex or of materials added to the latex which might conceivably act as precursors of rubber, the following preparations from *C. grandiflora* were tested as sources of enzymes in attempting rubber formation in vitro: Serum containing some rubber, rubber-free serum, purified serum proteins, whole-leaf cytoplasm, purified leaf-cytoplasm proteins, and chloroplasts from leaves of *C. grandiflora*.

The following substances, which have been suggested as theoretically possible rubber precursors, were tested in concentration series from 1 to 10 mg. per milliliter as additions to the preparations just named in attempting rubber formation in vitro: None (formation of rubber by the preparation alone), citral, β-methyl crotonaldehyde, tiglic acid (sodium salt), isovaleric acid (sodium salt), isoleucine, isovaleraldehyde, tiglaldehyde, phenol isolated from *Cryptostegia grandiflora* insolubles, triterpenol from *C. madagascariensis* latex, and various ones of those previously listed plus hydrogen peroxide.

In each case the formation of rubber after overnight incubation at room temperature was estimated by drying down the whole reaction mixture and extracting it with benzene, in some cases after a preliminary extraction with alcohol. Then 5 ml. of the benzene extract was pipetted into an equal volume of alcohol, and the turbidity of the solution was measured in a Coleman photofluorometer.

In no case was any suggestion of rubber formation in vitro found. These experiments included many of the possible rubber precursors suggested in the literature (4), as well as their closely related derivatives; in addition three possible sources of rubber-forming enzymes—namely, latex-serum proteins, leaf-cytoplasm proteins, and chloroplastic proteins—were used. The results were uniformly completely negative. Whether current theories of rubber formation are in error or an improper experimental approach was used cannot be stated.

SUMMARY AND CONCLUSIONS

Studies on latex physiology of *Cryptostegia grandiflora* have led to the following conclusions.

Latex flow from whips is influenced by relative humidity, wind, and temperature. The effects of these various environmental factors are interrelated. These factors appear to influence latex flow by affecting the water relations of the plant. Latex as it flows from a cut whip becomes progressively more dilute. In the course of exhaustive bleeding of a whip, sufficient rubber synthesis does not occur to maintain the concentration of rubber as found in the latex obtained in the initial bleeding. No reconcentration of the total solids in latex occurs in periods of 10 minutes to 96 hours after a bleeding cut is made. Of the total movement of latex in the first minute after a bleeding cut is made, 90 percent or more takes place within 40 inches of the bleeding cut.

Successive whip bleedings decrease both the yield of latex and the percentage of rubber in the latex. The total rubber yields by exhaustive whip bleeding may approach as much as 3 gm. per whip for 2- to 3-year-old plants. For 1½- to 2-year-old plants, in general, the yield is about 2 gm. A significantly higher yield of rubber is obtained by both bleeding and extracting a whip than by either process alone. No correlation between leaf rubber and the rubber content or yield of the whip latex was detected.

Latex can be coagulated by mechanical agitation; 98.2 percent of the coagulate can be accounted for by rubber (benzene-soluble), resins (acetone-soluble), and proteins. The remaining serum consists of 92.1 percent of water and 7.9 percent of solids. Of these solids all but 6.9 percent can be accounted for on the basis of known chemical fractions.

Whip latex obtained in the morning is higher in percentage of rubber and lower in percentage of insolubles than that obtained in the afternoon. The presence of foliage leaves on the whip being bled repeatedly is necessary for maintenance of the total-solids content of the latex. No rubber hydrocarbon can be detected in ungerminated seeds. The latex total solids from 3-week-old seedlings contain significant amounts of rubber. The solids of latex obtained from roots of mature plants contain as much as 30.7 percent of rubber. The rootstock does not influence the composition of the latex of the scion.

Tests with several possible enzyme preparations with several possible rubber-precursor substrates gave no indication of rubber synthesis in vitro.

LITERATURE CITED

(1) ALTMAN, R. F. A.
  1940. ORGANIC ANALYSIS OF HEVEALATEX. IV. AMINO-ACIDS. Arch. v. Rubbercult. Nederland. Indië 24: [647]-654. [In Dutch. English summary, p. 653.]
(2) ARISZ, W. H.
  1928. PHYSIOLOGIE VAN HET TAPPEN. [PHYSIOLOGY OF TAPPING.] Arch. v. Rubbercult. Nederland. Indië 12: [220]-241, illus.
(3) ARTSCHWAGER, E.
  1946. CONTRIBUTION TO THE MORPHOLOGY AND ANATOMY OF CRYPTOSTEGIA (CRYPTOSTEGIA GRANDIFLORA). U. S. Dept. Agr. Tech. Bul. 915, 40 pp., illus.

(4) BONNER, J., and GALSTON, A. W.
    1947. THE PHYSIOLOGY AND BIOCHEMISTRY OF RUBBER FORMATION IN
          PLANTS. Bot. Rev. 13:543–596.
(5) CURTIS, J. T., and BLONDEAU, R.
    1946. INFLUENCE OF TIME OF DAY ON LATEX FLOW FROM CRYPTOSTEGIA
          GRANDIFLORA. Amer. Jour. Bot. 33: 264–270, illus.
(6) FREY-WYSSLING, A.
    1932. INVESTIGATIONS ON THE DILUTION REACTION AND THE MOVEMENT
          OF THE LATEX OF HEVEA BRASILIENSIS DURING TAPPING. Arch.
          v. Rubbercult. Nederland. Indié 16: [285]–327, illus.
(7) ——
    1933. DER MILCHSAFTERGUSS VON HEVEA BRASILIENSIS ALS BLUTUNGSER-
          SCHEINUNG. EIN BEITRAG ZUR DRUCKSTROMTHEORIE. Jahrb. f.
          Wiss. Bot. 77: [560]–626, illus.
(8) HENDRICKS, S. B., and WILDMAN, S. G.
    1946. A TRITERPENE ESTER ISOLATED FROM CRYPTOSTEGIA MADAGASCARI-
          ENSIS LATEX. Arch. Biochem. 10: 157–162.
(9) —— WILDMAN, S. G., and McMURDIE, H. F.
    1944. MORPHOLOGY OF LATEX PARTICLES AS SHOWN BY ELECTRON MICRO-
          GRAPHS. India Rubber World 110: 297–300, illus.
(10) ITERSON, G. VAN, Jr.
    1936–37. RUBBER SYMPOSIUM AT DELFT. X—HEVEA BRASILIENSIS AS A
          PRODUCER OF RUBBER. India-Rubber Jour. 92 (26): 3–9
          (869)–(875), 1936; 93 (1): 11–18 (23)–(30); 93 (2): 10–15
          (60)–(65), illus., 1937.
(11) MOYER, L. S.
    1937. RECENT ADVANCES IN THE PHYSIOLOGY OF LATEX. Bot. Rev. 3:
          522–544.
(12) POLHAMUS, L. G., HILL, H. H., and ELDER, J. A.
    1934. THE RUBBER CONTENT OF TWO SPECIES OF CRYPTOSTEGIA AND OF AN
          INTERSPECIFIC HYBRID IN FLORIDA. U. S. Dept. Agr. Tech. Bul.
          457, 23 pp., illus.
(13) PUCHER, G. W., WAKEMAN, A. J., and VICKERY, H. B.
    1941. ORGANIC ACIDS IN PLANT TISSUES: MODIFICATIONS OF ANALYTICAL
          METHODS. Indus. and Engin. Chem., Analyt. Ed. 13: 244–246.
(14) SPENCE, D.
    1908. ON THE PRESENCE OF OXIDASES IN INDIA-RUBBER, WITH A THEORY
          IN REGARD TO THEIR FUNCTION IN THE LATEX. Biochem. Jour.
          3: 165–181.
(15) STEWART, W. S., and HUMMER, R. W.
    1945. INVERSE CORRELATION BETWEEN RUBBER HYDROCARBON AND A
          CRYSTALLINE FRACTION ISOLATED FROM LATEX OF CRYPTOSTEGIA
          GRANDIFLORA. Bot. Gaz. 106: 333–340, illus.
(16) SUMNER, J., and SOMERS, G. F.
    1943. CHEMISTRY AND METHODS OF ENZYMES. 365 pp., illus. New York.
(17) TRISTRAM, G. R.
    1941. THE PROTEINS OF HEVEA BRASILIENSIS. 2. ANALYSIS OF A PRODUCT
          ISOLATED FROM CREPE RUBBER. Biochem. Jour. 35: 413–416.
(18) WHITBY, G. S., and GREENBERG, H.
    1941. THE ISOLATION OF AMINO-ACIDS FROM RUBBER LATEX. Biochem.
          Jour. 35: 640–649.
(19) WHITTENBERGER, R. T., BRICE, B. A., and COPLEY, M. J.
    1945. DISTRIBUTION OF RUBBER IN CRYPTOSTEGIA AS A FACTOR IN ITS
          RECOVERY. India Rubber World 112: 319–323, illus.

SUSCEPTIBILITY OF GUAYULE TO VERTICILLIUM WILT AND INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON DEVELOPMENT OF INFECTION [1]

By HENRY SCHNEIDER [2]

Formerly *assistant pathologist, special guayule research project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

In the winter and spring of 1942 and 1943 extensive plantings of guayule (*Parthenium argentatum* A. Gray) were made in California. Although several diseases of guayule had already been described,[3] the seriousness of some of them, especially verticillium wilt, caused by *Verticillium albo-atrum* Reinke and Berth., was not fully realized. The results of later surveys on the occurrence of verticillium wilt and the losses caused by it have been reported,[4] and the disease has been discussed by Campbell and Presley (*5*).[5] This paper records the results of tests to determine (1) the susceptibility of eight commercial strains of guayule to verticillium wilt and (2) the influence of soil temperature and moisture on the development of infection. An abstract of the findings of the preliminary experiments has been published (*18*).

MATERIALS AND METHODS

The eight commercial strains [6] of guayule most commonly planted from 1942 to 1945 under the emergency rubber project program were grown at various places in California to test their reaction to verticillium wilt under different environmental conditions. The effect of soil temperature on the disease in strain 109 was studied in the greenhouse at Salinas, and the effect of soil moisture on the disease

---

---

when affecting strain 593 was studied in field plots at Shafter. The details of the experiments are presented in the sections in which the results are given.

The data were analyzed by Snedecor's analysis-of-variance methods (*19*). Because the data involved the number of diseased plants or of plants remaining healthy out of a given number observed, the values were changed to percentages, which were transformed to angles (*19, table 16.8*). When none or all of the plants observed had the particular attribute the original values were adjusted before they were changed to percentages by assuming, for instance, that 1/4 of 1 plant remained healthy when all were diseased and that only 9¾ of 10 plants remained healthy when 10 were observed to be healthy (Bartlett's correction (*1*)). The *F* test was applied to the mean squares obtained.

In some instances analyses were also run without either Bartlett's correction or transformation of the data. The *F* values obtained, but not used herein, were not appreciably different from those computed after the transformations. From the mean squares obtained from the analyses the mean differences required for significance between means of treatments were calculated. Although according to some authorities on statistical methods these values theoretically may not be as sensitive as those from transformed data, they can be used with the means from the original data rather than with means of angles, which are rather abstract.

SUSCEPTIBILITY OF COMMERCIAL STRAINS OF GUAYULE

The eight strains of guayule commonly planted may be loosely grouped into four main types on the basis of the external morphological characteristics which they have in common. Strain 109 comprises one type; 111 a second; 130, 406, and 593 a third; and 405, 407, and 416 a fourth. The number of chromosomes (*2*) and the sizes, dry weights, and resin and rubber content of these strains (*8*) have been studied. These strains reproduce mostly by apomixis; therefore, for practical purposes each may be considered an asexual clone (*7, 14*). For several reasons the strains used were not pure (*8*)[7]— the seed fields had not been rogued, there was some variation in reproduction (*2, 14*), and there may have been some mixing of seed. Strain 109 had the largest number of offtype plants. The typical plant of this strain was most susceptible to verticillium wilt; therefore, the injury reported is an underestimate for the typical plant of strain 109. Since the cause of variation was not known when the work was begun, it did not seem advisable to attempt to sort out the typical plants.

In early summer of 1943 parts of the approximately 30,000 acres of guayule planted in different parts of California were showing the effects of verticillium wilt. One of the most heavily infected fields, located near Shafter, was planted to strain 109, but through error two rows of strain 405 plants were also planted in this field (fig. 1). Most of the typical plants of strain 109 were severely stunted or killed, but the majority of those of strain 405 and many of the offtype plants of strain 109 showed little disease, although they were infected.

[7] Nursery plants or seeds were obtained from the Emergency Rubber Project.

Many plants of strains 406, 593, and 130 in other fields showed symptoms ranging from slight injury to killing at the same time, but usually plants of strains 405 and 407 were less severely affected. During the summer of 1943 in the warm interior valleys many diseased plants of all strains recovered. Later when the cool temperatures of fall were again favorable for the development of verticillium wilt, only the plants of strain 109 showed external symptoms.

Near Salinas a preliminary test of six commercial strains of guayule was made in a nursery-type variety planting (five randomized blocks) on soil infested with *Verticillium albo-atrum* and nematodes (*Heterodera marioni* (Cornu) Goodey). At the end of the growing season the taproots were cut to see what percentage had darkbrown discoloration of the wood and were therefore presumably

FIGURE 1.—Field of guayule strain 109 in which through error two rows of strain 405 had been planted, Shafter, Calif., July 1943. Note that plants of strain 109 were severely affected with verticillium wilt whereas those of strain 405 (center) were only slightly affected.

infected by *Verticillium*. The results were as follows: Strain 109, 74 percent; strain 593, 66 percent; strain 406, 56 percent; strain 130, 53 percent; strain 416, 47 percent; and strain 407, 42 percent. According to the F test the difference in susceptibility among strains was highly significant.

In the winter of 1943–44 field tests were begun in four localities. The designs were randomized blocks or Latin squares. Details of the experiments follow:

The Greenfield experiment was located in the middle of the Salinas Valley where soil temperatures favorable for activity of *Verticillium* prevailed all summer. The plots were planted December 31, 1943, and final data were taken September 18, 1944. As *Verticillium* was not uniformly distributed through this field, the differences between means are small and the differences required between means for significance are large (table 1).

Salinas experiment 1 was located in the lower Salinas Valley where soil temperatures favorable for activity of *Verticillium* prevailed all summer. The plots were planted June 26, 1944, and harvested March 27, 1945

TABLE 1.—*Average number of guayule plants out of 8 of each strain affected in varying degrees with verticillium wilt in field-type plantings, California*[1]

Location of planting	Degree of injury of plants	Type 1 (109)	Type 2 (111)	Type 3				Type 4		Amount for significance at—		F test
				130	406	593	405	407	416	5-percent level	1-percent level	
		Number	Number	Number	Number	Number	Number	Number	Number			
Greenfield[2]	Killed	2.6	2.2	0.9	0.1	0.3	0.3	0.4	0.1			
	Severely and moderately affected	1.7	1.6	2.4	2.1	3.7	1.0	.6	.6			
	Slightly affected and healthy	3.7	4.3	4.7	5.7	4.0	6.7	7.0	7.3	1.8	2.4	**
Salinas (trial 2)[3]	Killed	1.8		.7	.4	.4	.4	.9	.1			
	Severely and moderately affected	4.7		4.0	4.5	4.7	1.2	7.0	.3			
	Slightly affected and healthy	1.5		3.3	3.3	2.8	6.4	7.0	7.5	1.0	1.3	**
Salinas (trial 1)[4]	Killed						.6	.1	.1			
	Severely and moderately affected			2.5	2.4	2.1	7.1	.4	.4			
	Slightly affected and healthy			5.2	5.5	5.8	0	7.5	7.5	.9	1.2	*
Shafter[5]	Killed	0	0					.1	.3			
	Severely and moderately affected	3.1	1.6	.1	0	.3	.8	.4	.3			
	Slightly affected and healthy	4.9	6.4	7.9	8.0	7.8	7.3	7.5	7.6	.9	1.2	**

**Highly significant.
[1] For details concerning the plots see p. 131.
[2] Each value is an average for 7 plots.
[3] Each value is an average for 12 plots.
[4] Each value is an average for 15 plots.
[5] Each value is an average for 8 plots.

Salinas experiment 2 was adjacent to Salinas experiment 1. The plots were planted April 1, 1944, and harvested August 21, 1944.

The Shafter experiment was located near Shafter. The plots were planted November 25, 1943, and the final data were taken February 27, 1945. Summer air temperatures were unfavorable for the disease. (See fig. 3.) Since irrigating tends to cool the soil, the plots were irrigated weekly in an attempt to keep the soil temperature within a range favorable for verticillium wilt. This experiment and another in the same locality were established on land on which guayule had been severely diseased the previous year. Although spring temperatures in both years were favorable for disease activity, symptoms were not severe in either plot the second year, indicating possibly that *Verticillium* does not build up in soil planted to guayule. Injury was not so severe in this locality as in others where temperatures were more favorable for the fungus.

In each plot eight plants chosen at random were graded according to injury from *Verticillium.* The symptoms were observed monthly; at the end of the experiments the plants were pulled and the taproots were cut to see whether there were any dark-brown discolorations in the wood. In two of the experiments the rubber yields also were determined. The data in tables 1 and 2 indicate that degree of injury and reduction in yields were correlated. It had been found previously [8] that plant size was reduced by verticillium wilt but that on the dry-weight basis the percentage of rubber was the same in injured plants as in healthy ones.

TABLE 2.—*Rubber yield per acre of guayule strains grown on Verticillium-infested soil, California*

[8 plants harvested per plot]

Strain	Yield per acre at—	
	Greenfield [1]	Salinas (trial 1) [2]
	Pounds	*Pounds*
109	25	
111	25	
130	35	42
406	35	42
593	34	44
405	43	54
407	43	57
416	42	
Difference required for significance:		
5-percent level	14.4	9.5
1-percent level		12.6

[1] Mean of 7 replicates of 8 plants of each strain. The differences are not significant according to the F test.

[2] Mean of 15 replicates of 8 plants of each strain. The differences are highly significant according to the F test.

It may be concluded that strain 109 is very susceptible to verticillium wilt; strains 130, 406, and 593 are moderately affected; and strains 405, 407, and 416 are affected least, though occasional plants may be killed. Differences in susceptibility between the last two groups did not occur in the Shafter trial probably owing to unfavorable summer temperatures. Strains within morphologically similar groups of plants were for the most part similar in resistance to the disease. It is interesting to note that strains which yield best in the absence of the disease (*8*) yield least when the disease is present.

―――――――――

[8] See footnote 4, p. 129.

SOIL-TEMPERATURE STUDIES

The effect of soil temperature on verticillium wilt has received considerable attention. Ludbrook (*12*) tested cultures from *Verticillium*-diseased plants from various parts of the world, including several from California. He found in culture that one group of fungi which he designated as *Verticillium albo-atrum* R. and B. had a lower maximum temperature for growth than a microsclerotium-producing group which he designated as *V. dahliae* Klebahn. He found that growth of *V. albo-atrum* was greatly retarded at 82° F. and none occurred at 86°. *V. dahliae* grew well at 82° and all cultures of it made some growth at 86°. The results obtained when plants were grown in infested soil at controlled temperatures indicated that these fungi had the same maximum temperatures in plants as in cultures. McKeen (*13*) found that the microsclerotium-producing fungus designated as *V. albo-atrum* produced its maximum damage in tomatoes at a soil temperature of 75° and became inactive at about 86°. Williams (*20*) recently found that in culture *V. dahliae* is favored by slightly higher temperatures than *V. albo-atrum*.

The microsclerotium-producing fungus here designated as *Verticillium albo-atrum* segregated into microsclerotium- and non-microsclerotium-producing cultures on single sporing, as isolates from other diseased agricultural crop plants had previously been found to do (*15*). Two isolates were tested—one from near Salinas, where moderate temperatures prevail, and the other from near Shafter, where temperatures are presumably too high to favor the development of *V. albo-atrum* in the summer.

The soil in which these isolates were tested was maintained at constant temperatures ranging from 51° to 105° F. in cans suspended in tanks of water like those described by Campbell and Presley (*4*). The cans were 18 inches high. The lower 4 inches was filled with sand; above this was 8 inches of sandy loam. About 200 cc. of oat cultures per can was mixed with the soil.[9] No sterile oats were used in the control cans. A space of 6 inches was left above the soil surface to provide warm air over the soil, and the top of the soil was covered with glass wool to minimize temperature changes at the soil surface. Glass tubes were inserted on the sides of the cans so that any accumulation of water at the bottom of the cans could be detected. Two plants of strain 109 which had been grown from seed in 2-inch pots were planted in each can. Three cans containing soil infested with the fungus from near Shafter, three containing soil infested with the fungus from Salinas, and two control cans containing uninfested soil were used at each temperature. Soil temperatures were taken morning and night. The averages of all the readings, together with the dry weights of the plants determined after 9 weeks, are given in figure 2. The weights shown are the average weights of plants per can from two cans selected at random from each set of three cans. The check data are the average weights of plants per can from the two control cans. The plants made practically no growth at 51° and 105°, and apparently the infected plants were stunted at temperatures between 72° and 79°. At temperatures of 85° and above no infection occurred, but the oat medium seemed to affect fertility.

---

[9] The medium for the cultures was prepared by boiling whole oats.
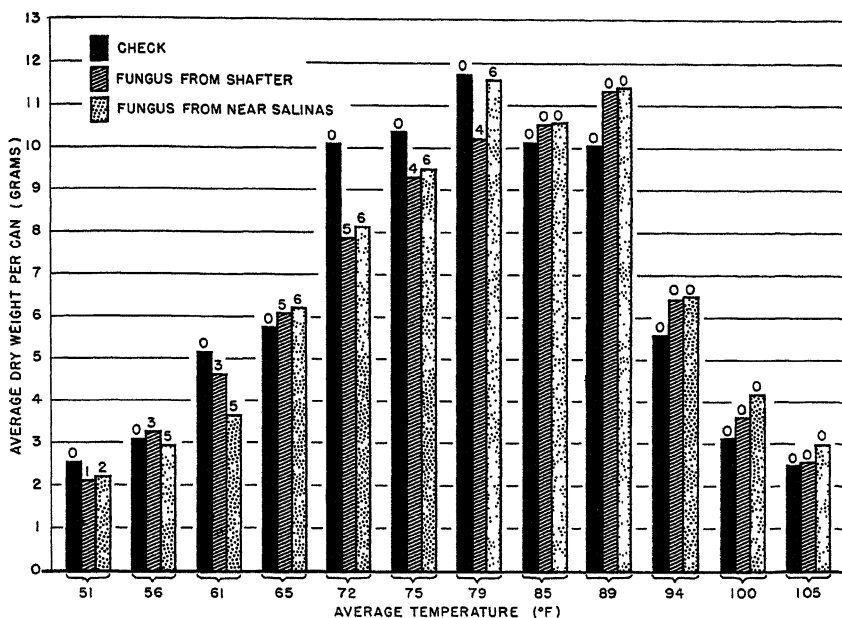
FIGURE 2.—Dry weight of plants grown in infested soil and in clean soil at various temperatures. The numbers above the bars indicate how many of the six plants in each series were infected.

Summer temperatures in the Salinas Valley are considered optimum for growth of *Verticillium*, but those in Kern County are believed to be unfavorable for its development during the warm summer months. For ease of comparison air and soil temperatures for Salinas and air temperature for Bakersfield, which is near Shafter and has about the same temperature, are presented graphically in figure 3.   Soil temperatures for Shafter are shown in figure 4.

EFFECTS OF SOIL MOISTURE AT SHAFTER

The study of the relation between soil moisture and verticillium wilt brings up two questions: (1) What is the relation between soil
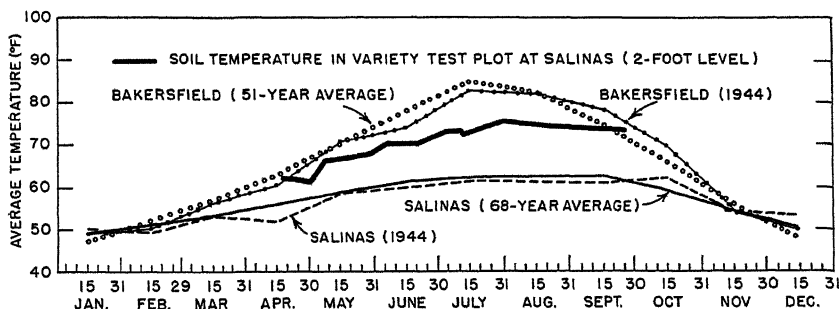
FIGURE 3.—Monthly means of each day's mean air temperature for Bakersfield, Calif., which is near Shafter and has about the same temperature, and for Salinas, Calif., and soil temperatures at the 2-foot level at Salinas. (Air-temperature data from the U. S. Weather Bureau.)

moisture and the number of infections by *Verticillium*? (2) Which moisture tensions favor the disease once the plants are infected?

Literature published before 1931 on soil moisture as related to verticillium wilt was reviewed by Rudolph (*17*). McKeen (*13*) found that when *Verticillium* inoculum was placed in dry soil and fallowed for 2 months before cropping, the fungus took longer to attack the plants than when inoculum was so placed in wet soil. Rada (*16*) reported that infection of young cotton plants of one of the three varieties tested did not occur so soon when the first irrigation was delayed for 2 months after planting as when it was delayed only 1 month. All subsequent irrigations were the same for both treatments. Infections were determined by observing the discolorations in the vascular bundles of leaf petioles. As the treatments were not replicated, the observed delay in infection may well have been due to lighter infestation of the soil. Final counts determined by the presence of discoloration of the vascular tissue of the stem at the ground level indicated no differences due to treatments. No reference was made to rainfall or soil moisture.

Ludbrook (*12*), in greenhouse tests with eggplants, attempted to maintain soil at various percentages of the moisture-holding capacity (a condition of soil moisture brought about by empirical methods described by Riker and Riker [10] and somewhat above field capacity) by weighing the pots at intervals and adding enough water to bring the ratio of water to soil to some desired percentage. In such cases, as Hendrickson and Veihmeyer (*9*) have found, added water wets only a portion of the soil. For this reason no actual difference in water tension between Ludbrook's treatments occurred, but the top layers of soil in all treatments were maintained at the same degree of wetting. Therefore his conclusion that soil moistures between 45 and 95 percent of the moisture-holding capacity had no appreciable effect on verticillium wilt of eggplant except at 82.4° F. (at which a lengthening of the incubation period and a reduction in severity occurred in his highest two soil-moisture treatments) appears to be invalid. Isaac (*11*) also studied the effect of soil moisture on the disease in sainfoin plants by adding enough water at intervals to bring the soil to various percentages of the water-saturation point. He gave no data on the field capacity or wilting point of the soil. His results showed that "an increase in the water content of the soil decreases the incidence of disease" (*11, p. 33*). Apparently the soil moistures which Ludbrook and Isaac found to be adverse to the disease were above field capacity and would not be found in the field in well-drained soils for any length of time after irrigations. Perhaps the effect produced was caused by limitation of the aeration.

1943 IRRIGATION EXPERIMENT

In August 1943 an experiment was set up in a field moderately affected with wilt near Shafter, Calif. The following arbitrary irrigation treatments were used: (1) Irrigation every week (water applied

[10] RIKER, A. J., and RIKER, R. S. INTRODUCTION TO RESEARCH ON PLANT DISEASES— A GUIDE TO THE PRINCIPLES AND PRACTICE FOR STUDYING VARIOUS PLANT-DISEASE PROBLEMS. 117 pp., illus. St. Louis, Chicago, etc. 1936. [Processed.]

for 30 minutes after it reached the ends of the furrows) ; (2) irriga-
tion every 2 weeks (water applied for 1 hour after it reached the ends
of the furrows) : (3) irrigation every 4 weeks (water applied for 2
hours after it reached the ends of the furrows) ; and (4) no irrigation.
The last field irrigations before part of the field was taken over for
the experimental plots was about July 1. The experimental irriga-
tions were begun on August 6 when water was applied in treatments
1, 2, and 3. The last irrigation was made on October 22. The experi-
ment was set up on a well-drained Hesperia sandy loam with the wilt-
ing point ranging from 7 to 10 percent and the field capacity from
15 to 20 percent depending on the location and depth of the soil.
The treatments were replicated in 5 randomized blocks. For each
plot 20 plants were selected—5 healthy, 5 slightly affected, 5 moder-
ately affected, and 5 severely affected. The condition and growth of
these plants were observed each month.

Soil-moisture determinations by the oven-dry method were made
every 2 weeks on the day before irrigation to the 4-foot level for each
plot. No determinations were made immediately after the irrigations,
but penetration as a result of short irrigations in this type of soil
when it was at the wilting point was 1 to 2 feet. The average soil-
moisture conditions for the first 4 feet in the plots follow.

Of the plots irrigated at 1-week intervals that in block A remained
above 15 percent at all levels. Between irrigations plots in the other
blocks did not dry quite down to the wilting point in the upper 2 feet,
and after the middle of October the upper 2 feet of soil dried to only
about 15 percent. The water did not penetrate beyond the 2-foot level
in these four plots, and the soil was near the wilting point at the
lower depths.

In all except three of the plots irrigated at 2-week intervals the soil
approached the wilting point when sampled. In these three plots
the moisture was slightly above the wilting point between the 2- and
4-foot levels during August and the first half of September.

In the plots irrigated at 4-week intervals the soil approached the
wilting point within 2 weeks after irrigation at all levels except the
2- to 4-foot levels during August and the first half of September.

All the dry plots were at the wilting point after August 20 except
those in three of the blocks in which the soil at the 2- to 4-foot levels
did not reach the wilting point until about the end of September.

On April 1, 1944, for the plants which were apparently healthy on
August 6, 1943, the number of infections was determined by cutting
the taproots. On the plots irrigated every week 77 percent of the plants
were infected by *Verticillium;* on those irrigated every 2 weeks, 62
percent; on those irrigated every 4 weeks, 42 percent; and on those
left dry, 8 percent. The *F* test indicates highly significant differences.
In the dry plot occasional plants that showed recovery from previous
infection by *Verticillium*, but that were not detected by cutting
branches on August 6, were recorded as healthy. On one plot two
plants and in each of four others one plant had died from causes other
than infection by *Verticillium*.

From the results obtained from these plots the following general
conclusions seemed justified : Frequent irrigations favored infection by
Verticillium. On the average, moderately and severely affected plants

remained so in dry plots, but such plants showed about the same degree
of improvement in each of the three irrigated treatments. Since tem-
peratures were unfavorable for the disease during August and Sep-
tember, recovery was probably more from the severe injury that
resulted from the spring epidemic than from active verticilliosis.
Slightly affected plants responded like healthy ones regardless of the
type of treatment. In dry plots growth as indicated by plant spread
and dry weight was greatly reduced; rubber content, however, was
increased.

1944 IRRIGATION EXPERIMENT

In 1944 an attempt was made to prevent infection by keeping the
upper layers of soil—where *Verticillium* was assumed to occur in the
greatest concentration—near the wilting point at times when tem-
peratures were favorable for infection. Four treatments replicated
five times were set up on a different part of the field that had been
used for the 1943 experiment. The ground chosen was heavily in-
fested. Guayule plants set out the previous year were removed, and a
new planting of strain 593 was made. The ground was wet to the
7-foot level by irrigations before and during planting.

Treatment 1 consisted of irrigation of the soil for 2 hours each week.
Plaster of paris blocks described by Bouyoucos and Mick (*3*) for de-
termining soil moisture were placed at 1-, 3-, and 5-foot depths.
Treatment 2 consisted of irrigation after the 1-foot level of soil had
been at the wilting point for 1 week. The plots were irrigated until
the profile was wet to 6 feet. Since irrigating was done only once
a week, the plots were irrigated as soon as possible after the 1-week
period had elapsed, but not exactly at the end of 1 week. Plaster of
paris blocks were placed at 1-, 2-, 3-, 5-, and 7-foot depths. Treatment
3 was the same as treatment 2 except that no irrigations were given
when the temperature of the soil was below 80° F. at the 18-inch level.
In treatment 4 no irrigating was done after the plants became estab-
lished. In treatments 3 and 4 plaster of paris blocks were placed at
the same levels as in treatment 2.

Readings of 60,000- to 75,000-ohm resistance in the plaster of paris
block were considered to indicate that the soil was at the wilting point
(*3*).

The plots were planted during the second week of March in rows
28 inches apart and with plants 20 inches apart in the rows. It was
hard to get the plants to grow, and nongrowers were replanted on
March 30, May 17, and June 9. The replants were watered by hand.

SPRING INFECTIONS

On May 18, 15 vigorous plants, each having at least 3 neighbors,
were selected and staked in each plot for further observation. For
final analysis 10 of these not affected by other causes were selected
at random. It was soon obvious that the water was not being with-
drawn from the soil rapidly enough to bring about the desired dif-
ferences in treatments during the period of favorable temperatures
in spring. Therefore, the original design was modified for the
spring period by irrigating every 2 weeks instead of every week

in treatment 2. As indicated in figure 4, there was little difference between the treatments in the number of infections that occurred, but the number of infections was significantly greater in plots irrigated weekly than in the plots of the two treatments that had not received water since the treatments began.

Injury to plants in the spring was not severe, possibly because the inoculum potential did not build up in the soil when it was cropped to guayule the previous year. Temperatures were very favorable for *Verticillium* in the spring of 1943 and 1944, but in the plants chosen for observation the symptoms were slight except in a few that were moderately affected. There were no differences between treatments.
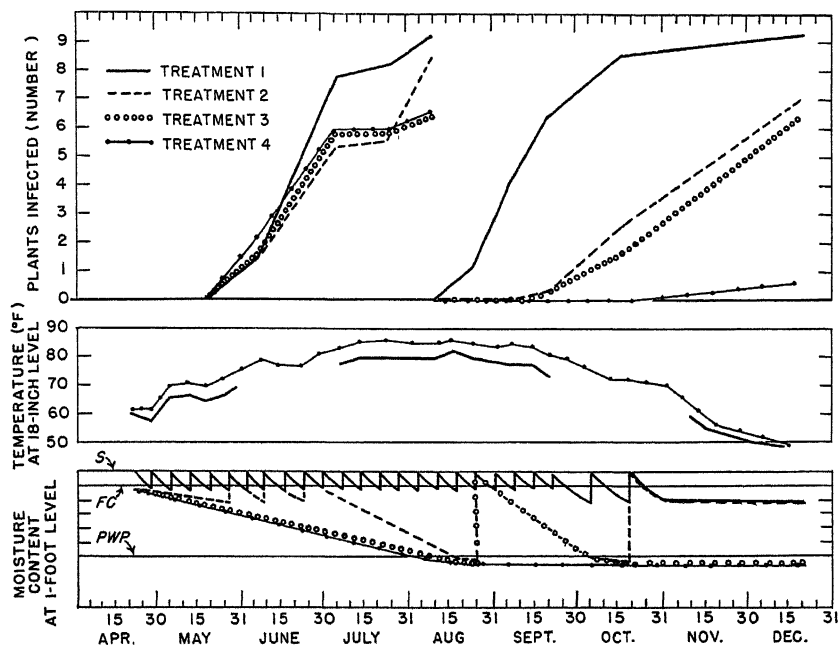
FIGURE 4.—Relation of soil temperature and soil moisture to verticillium wilt of 10 plants chosen as healthy on May 18, 1944, and of 10 chosen as healthy on August 9 in 5 randomized blocks given various irrigation treatments, Shafter, Calif. For the August 10 readings at the 5-percent level 1.7 plants were required for significant differences between means of the May plants, and for the December 20 readings 4.2 plants were required for significant differences between means of the August plants; at the 1-percent level 2.2 and 5.9 plants, respectively, were required. *FC*, field capacity (approximated); *PWP*, permanent wilting point; *S*, saturation point.

Yields from the plants selected on May 18 in the various plots were determined on December 20 (table 3). Withholding irrigations caused a highly significant increase in rubber content and a highly significant decrease in dry weight. The yields were lowered by withholding water, but not significantly so. This inverse relation of rubber production and shrub weight was also found by Hunter and Kelley (*10*) in healthy plants on a Delano sandy loam near Shafter.

TABLE 3.—*Effect of irrigation treatments on rubber content, dry weight, and yield of guayule plants in 5 randomized blocks collected Dec. 20, 1944, Shafter, Calif.*

[Each value based on a composite sample of 10 plants from each plot]

Treatment No.	Rubber content [1]	Average dry weight per plant [1]	Rubber yield per acre [2]
	Percent	*Grams*	*Pounds*
1	3 00	153.9	113.7
2	3.01	148.4	110.8
3	3.46	125.2	106.2
4	4.47	84.6	92.9
Difference required for significance.			
5-percent level	.39	22.1	16.7
1-percent level	.55	31.0	23.4

[1] Differences highly significant according to *F* test.
[2] Differences not significant according to *F* test.

FALL INFECTIONS

On August 9, 10 apparently healthy plants (mostly replants) of equal size and having 4 neighbors were chosen in each plot and staked. Temporary spring treatments were discontinued on July 7 and those originally outlined were begun. The occurrence of infection as related to temperature and moisture are shown in figure 4. For the December 20 data plants were pulled and the taproots were cut to determine infection. For other dates the branches were cut on plants exhibiting possible top symptoms. Moisture data for the 1-foot level are presented in figure 4. The rate at which the lower levels reached the wilting point was related to the treatments. In treatment 2 in August the wilting point was reached at the 2-foot level within 10 days after it was reached at the 1-foot level in 1 replicate; the irrigation was made before the wilting point was reached at the 2-foot level in the other 2 replicates in which plaster of paris blocks were used. Only 1 replicate had reached the wilting point at the 2-foot level at the time of the October irrigation. In treatment 3 only 1 replicate had reached the wilting point at the 2-foot level at the time of the August 25 irrigation. The same replicate was at the wilting point again at the 2-foot level on October 17, and the other 2 had reached the wilting point at the 2-foot level by November 10. One replicate had reached the wilting point at the 3-foot level by December 20. In treatment 4 the wilting point at the various depths was reached on the following dates: Replicate A: 1-foot level, August 24; 2-foot level, August 24; 3-foot level, October 17; 5-foot level, December 20. Replicate C: 1-foot level, July 24; 2-foot level, August 26; 3-foot level, September 7; 5-foot level, October 17; 7-foot level, not reached. Replicate E: 1-foot level, July 24; 2-foot level, August 24; 3-foot level, September 20; 5- and 7-foot levels, not reached.

Continued low soil-moisture tension appeared to favor infection (treatment 1), but when the upper layers of soil, in which the fungus is probably most extensively distributed, were maintained at the wilting point few infections occurred (treatment 4). Frequent irrigations lowered the soil temperature; as a result, infection occurred during the summer in treatment 1. Drying the upper layers of soil to the wilting point between irrigations in treatments 2 and 3 had no significant effect on the final readings as compared with those in

treatment 1, but it did delay infection until the temperature began to moderate in the latter part of September. Infections occurred in treatment 3 when the soil moisture was somewhat below the field capacity but above the wilting point.

DISCUSSION

Because of the nature of guayule there are several possible methods of reducing losses from verticillium wilt. Guayule differs from most agricultural crops in that it is a desert plant, which can withstand severe drought. In fact, yields of rubber (pounds per acre) may be as high, depending on soil type, when plants are grown under high soil-moisture tension as when grown under low tension (*10*). The reason is that the percentage of rubber formed increases with an increase of moisture tension.

In a climate like that of Shafter, Calif., after plants are once established in the field, some degree of control may possibly be effected by allowing the upper 2 or 3 feet of soil to remain at the wilting point as long as possible, especially when temperatures are favorable for the growth of *Verticillium*. However, while plants are being established this method of control is not possible. There are several reasons for this. In the first place, it is necessary to maintain low soil-moisture tension to establish plants (*6*), and it takes several months after plants begin growth for them to develop tops and root systems large enough to dry the soil to the wilting point. Moreover, the temperature must be sufficiently high to cause the plants to begin growth but not so high as to cause rapid drying of the top 6 inches of soil. Such moderate temperatures also favor the growth of *Verticillium*. However, some plants escape infection during this period of establishment, possibly because their root systems are not sufficiently extensive to reach the fungus. Many other plants are only slightly affected and recover. That woody plants recover from verticillium wilt is well known. Recovery occurs in both guayule and trees such as apricot, in which rings of discolored wood are found only in occasional years. Each discolored ring is presumably the result of a new infection. In guayule the cell walls of infected wood turn dark brown, and the vessels become filled with a brown amorphous substance. This material may cause the death or isolation of the fungus in affected vessels. There are also some indications that multiple infections are more serious than a single infection. The only decided reduction in infection in the experiment described here occurred when the soil was maintained at the wilting point.

Once plants are established and are large enough to withdraw water from the soil rapidly, certain irrigation practices may be beneficial in preventing further infections. In the interior valleys of California where high summer temperatures are unfavorable for the growth of the fungus, it seems desirable to irrigate only in summer provided there is no danger from phytophthora root rot or drowning, which occurs when irrigation, high temperatures, and certain other conditions are combined (*5*). When winter irrigating is done, the water may not be completely withdrawn from the upper layers of soil until new growth begins in spring. Then it is gradually withdrawn. Since the fungus also becomes active at this time, the maximum number of infections occurs. Plants of strain 593 beginning their second year's

growth in the field have shown severe discoloration of the taproots under these conditions. Top symptoms, however, were not noticed in this strain, though stunting of the plants no doubt occurred.[11]

The strain tests have shown that when the fields to be used are known to be infested with *Verticillium*, it is essential that very susceptible strains be avoided.

SUMMARY

Experiments conducted in California from 1942 to 1945 indicated that the commercial strains of guayule differ in susceptibility to *Verticillium albo-atrum*. Plants of strain 109 are very susceptible to verticillium wilt and may be killed at any age. Less extensive observations and tests indicate that strain 111 is similar to 109 in susceptibility. Strains 405, 407, and 416 are the most resistant, but occasional plants may be killed by verticillium wilt when young. Strains 130, 406, and 593 are intermediate in resistance.

Experiments in constant-temperature tanks indicated that *V. albo-atrum* becomes inactive at soil temperatures between 80° and 85° F. Irrigation experiments at Shafter, Calif., in field trials seem to show that this fungus is active in soil at all moisture levels above the wilting point. Maintaining the upper soil horizons at the wilting point prevents infection.

LITERATURE CITED

(1) BARTLETT, M. S.
   1947. THE USE OF TRANSFORMATIONS. Biometrics Bul. 3: 39–52.
(2) BERGNER, A. D.
   1946. POLYPLOIDY AND ANEUPLOIDY IN GUAYULE. U. S. Dept. Agr. Tech. Bul. 918, 36 pp., illus.
(3) BOUYOUCOS, G. J., and MICK, A. H.
   1940. AN ELECTRICAL RESISTANCE METHOD FOR THE CONTINUOUS MEASUREMENT OF SOIL MOISTURE UNDER FIELD CONDITIONS. Mich. Agr. Expt. Sta. Tech. Bul. 172, 38 pp., illus.
(4) CAMPBELL, W. A., and PRESLEY, J. T.
   1945. DESIGN FOR CONSTANT-TEMPERATURE TANKS. Phytopathology 35: 213–216, illus.
(5) ——— and PRESLEY, J. T.
   1946. DISEASES OF CULTIVATED GUAYULE AND THEIR CONTROL. U. S. Dept. Agr. Cir. 749, 42 pp., illus.
(6) ERICKSON, L. C.
   1945. THE WATER FACTOR IN TRANSPLANTING GUAYULE. Amer. Jour. Bot. 32: 634–643, illus.
(7) ESAU, K.
   1946. MORPHOLOGY OF REPRODUCTION IN GUAYULE AND CERTAIN OTHER SPECIES OF PARTHENIUM. Hilgardia 17: [61]–120, illus.
(8) FEDERER, W. T.
   1946. VARIABILITY OF CERTAIN SEED, SEEDLING, AND YOUNG-PLANT CHARACTERS OF GUAYULE. U. S. Dept. Agr. Tech. Bul. 919, 25 pp.
(9) HENDRICKSON, A. H., and VEIHMEYER, F. J.
   1941. MOISTURE DISTRIBUTION IN SOIL IN CONTAINERS. Plant Physiol. 16: 821–826, illus.
(10) HUNTER, A. S., and KELLEY, O. J.
   1946. THE GROWTH AND RUBBER CONTENT OF GUAYULE AS AFFECTED BY VARIATIONS IN SOIL MOISTURE STRESSES. Amer. Soc. Agron. Jour. 38: 118–134, illus.
(11) ISAAC, I.
   1946. VERTICILLIUM WILT OF SAINFOIN. Ann. Appl. Biol. 33: 28–34, illus.

___

[11] See footnote 4, p. 129.

(12) LUDBROOK, W. V.
1933. PATHOGENICITY AND ENVIRONAL STUDIES ON VERTICILLIUM HADROMYCO-
SIS. Phytopathology 23: 117–154, illus.
(13) McKEEN, C. D.
1943. A STUDY OF SOME FACTORS AFFECTING THE PATHOGENICITY OF VERTI-
CILLIUM ALBO-ATRUM R. & B. Canad. Jour. Res., Sect. C, 21: |95]–
117, illus.
(14) POWERS, L., and ROLLINS, R. C.
1945. REPRODUCTION AND POLLINATION STUDIES ON GUAYULE, PARTHENIUM
ARGENTATUM GRAY AND P. INCANUM H. B. K. Amer. Soc. Agron. Jour.
37: 96–112.
(15) PRESLEY, J. T.
1941. SALTANTS FROM A MONOSPORIC CULTURE OF VERTICILLIUM ALBO-ATRUM.
Phytopathology 31: 1135–1139, illus.
(16) [RADA, G. G.]
1939. INFLUENCIA DEL RIEGO EN EL "WILT" DEL ALGODONERO. Lima, Peru,
Estac. Expt. Agr. de La Molina, Mem. 11a: 233–240, illus.
(17) RUDOLPH, B. A.
1931. VERTICILLIUM HADROMYCOSIS. Hilgardia 5: [197]–360, illus.
(18) SCHNEIDER, H.
1944. VERTICILLIUM WILT OF GUAYULE. (Abstract) Phytopathology 34:
936.
(19) SNEDECOR, G. W.
1946. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND
BIOLOGY. Ed. 4, 485 pp., illus. Ames, Iowa.
(20) WILLIAMS, P. H.
1944. VERTICILLIUM WILT. [Cheshunt] Expt. and Res. Sta. Ann. Rpt.
(1943) 29: 29–33.

SUCROSE, DEXTROSE, AND LEVULOSE CONTENT OF SOME DOMESTIC VARIETIES OF SORGO AT DIFFERENT STAGES OF MATURITY [1]

By EMIL K. VENTRE, *senior chemist*, S. BYALL, *associate chemist*, and J. L. CATLETT, formerly *junior sugar technologist, Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

Berthelot and Trannoy [2] studied the sucrose, dextrose, and levulose content of the juices of one variety of sorgo at eight stages of growth selected at about 14 days apart. They found that the dextrose content was consistently greater than the levulose content and both reached a minimum at the stage of maturity at which the sucrose content reached a maximum.

Willaman, West, Spriestersbach, and Holm,[3] after a study of three varieties of sorgo, reported that the amount of dextrose exceeded the amount of levulose throughout the growth of the plant, and they remarked that this characteristic of sorgo is different from that of most plant juices in which levulose generally is found to exceed dextrose. In general their results agree with those of Berthelot and Trannoy.

Ventre, Byall, and Walton,[4] in a study of the crystallization characteristics of sorgo sirups, noted that sirups made from immature canes and from the lower portions of the stalks crystallized dextrose, whereas the crystallization of sucrose occurred in sirups made from the upper portions of the stalk and from more mature canes. These facts suggested that by separation of the cane into two parts and working each separately one part would furnish on crystallization a source of sucrose and the other a source of dextrose. These facts further suggested that some varieties of sorgo might contain a higher content of dextrose than the four varieties used by Ventre and his associates.

MATERIALS AND METHODS

To supplement the previous studies and to obtain data on the principal varieties of sorgo (*Sorghum vulgare* Pers.) grown in the United States, the work described in this paper was carried out in cooperation with the Division of Sugar Plant Investigations, Bureau of Plant In-

[2] BERTHELOT, D., and TRANNOY, R. SUR LA TENEUR EN SUCRE, DU SORGHO AUX DIVERS STADES DE SA VEGETATION. [Paris] Acad. des Sci. Compt. Rend. 166: 824–827. 1918.
[3] WILLAMAN, J. J., WEST, R. M.. SPRIESTERSBACH, D. O., and HOLM, G. E. NOTES ON THE COMPOSITION OF THE SORGHUM PLANT. Jour. Agr. Res. 18: 1–31, illus. 1919.
[4] VENTRE, E. K., BYALL, S., and WALTON, C. F., JR. JELLYING AND CRYSTALLIZATION OF SIRUPS MADE FROM DIFFERENT PARTS OF THE SORGO STALK AT DIFFERENT STAGES OF MATURITY. Jour. Agr. Res. 59: 139–150. 1939.

Journal of Agricultural Research
Washington, D. C.

Vol. 76, Nos. 5, 6
March 14, 15, 1948
Key No. E-102

(145)

dustry, Soils, and Agricultural Engineering, United States Department of Agriculture. Thirty-four known varieties of sorgo were grown at the field station of that division near Meridian, Miss. The whole sorgo cane was cut and stripped, the seed heads were removed, and the cane was milled as soon as possible on a power-driven farm sirup mill which gave about 60 percent juice extraction. As far as it was possible to do so, each variety was studied in the three principal stages of maturity as denoted by the seeds being in the milk, dough-to-ripe, and dead-ripe stage. The juices expressed after being strained through a 300-mesh sieve were subjected to the following analytical determinations.

The concentration of dissolved solids or specific gravity in degrees Brix was determined on the freshly strained juice by a Brix hydrometer calibrated at 20° C.

Sucrose polarization, total reducing sugars, dextrose, and *levulose.*— A portion of each lot of freshly strained juice equivalent to five normal weights (26 gm.) was transferred to a 250-cc. volumetric flask, 12 cc. of a saturated neutral lead acetate solution was added, and the solution was made up to the mark and filtered.

(a) *Reducing sugars.*—To 50 cc. of the filtrate in a 200-cc. volumetric flask, 1 cc. of McAllep-Cook's solution, containing 7 gm. of disodium phosphate and 3 gm. of potassium oxalate to 100 cc., was added for each gram of total dissolved solids taken; the solution was made up to the mark and filtered; and the total reducing sugars were determined by the Lane-Eynon method.[5]

(b) *Polarization.*—Determinations were made by reading a deleaded portion of the filtrate in a jacketed 400-mm. tube in a saccharimeter at 20° C.

(c) *Sucrose (Clerget).*—Determinations were made on the filtrate according to the official method of the Association of Official Agricultural Chemists[6] with invertase as the inverting agent.

(d) *Sucrose, by copper reduction.*—The total quantity of reducing sugars was determined by the Lane-Eynon method[7] on the inverted solutions from the sucrose (Clerget) determination. Sucrose was calculated from the difference between the total reducing sugars as invert sugar determined before, and total reducing sugars as invert sugar determined after, inversion with invertase.

(e) *Levulose.*—The invertase-inverted solution from the sucrose (Clerget) determination was polarized in a metal-jacketed, 400-mm. polariscope tube at a temperature interval of about 60° C., and the total levulose content of the solution calculated from the factors determined by Jackson and Mathews.[8] The total levulose content of the inverted solution of the juice, corrected for the amount of levulose formed in the inversion of sucrose determined from the sucrose by copper reduction, was taken as the original levulose content of the juice.

---

[5] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. . . . Ed. 5, 757 pp., illus. Washington, D. C., 1940. (See pp. 498–499.)
[6] See pp. 491–493 of reference cited in footnote 5.
[7] See pp. 498–499 and 683–684 of reference cited in footnote 5.
[8] JACKSON, R. F., and MATHEWS, J. A. SOME PHYSICAL PROPERTIES OF LEVULOSE AND ITS ESTIMATION BY COPPER REDUCTION METHODS. [U. S.] Natl. Bur. Standards Jour. Res. 8: 403–444, illus. 1932.

Dextrose was determined by calculation from the foregoing determinations as follows: Total reducing sugars as invert sugar on uninverted juice (a) minus the levulose content of the original juice as determined under (e) calculated as invert sugar gives the dextrose content of the original juice in terms of invert sugar and is translated into dextrose by the Lane-Eynon tables [9] for given conditions.

PRESENTATION AND DISCUSSION OF RESULTS

Tables 1, 2, and 3 give results of the analyses of the sorgo juices at the three stages of maturity.

Examination of the tables shows that:

(1) The sucrose, dextrose, and levulose content of the juices vary widely between varieties.

(2) The total reducing sugars and dextrose and levulose content are highest in the immature stages and decrease with maturity. In the milk stage of maturity (table 1), the total reducing sugars as invert sugar exceed the sucrose content in varieties Nos. 3, 6, 7, 8, 12, 14, 15, 16, 25, 26, and 27. The dextrose content exceeds the sucrose content in varieties 3, 6, and 27.

(3) In agreement with previous observations, the sucrose content shows large increases with maturity, and the total of reducing sugars,

TABLE 1.—*Analysis of juices of sorgo at the milk stage of maturity*

No.	Variety	Dissolved solids by Brix hydrometer	Sucrose by polarization	Apparent purity	Sucrose by—		Reducing sugars as invert sugar	Dextrose	Levulose
					Clerget method	Copper reduction			
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	S. A. 287	15.61	10.95	70.14	10.98	11.20	1.62	0.92	0.64
2	Red X	16.12	11.10	68.85	11.15	11.33	2.27	1.03	1.17
3	Indiana Amber	18.04	4.10	22.72	5.78	5.62	9.88	6.47	3.12
4	S. A. 108	14.89	6.15	41.30	7.12	7.41	4.37	2.16	1.98
5	Straight Neck	18.05	8.60	47.64	9.78	9.69	5.92	2.62	3.12
6	Gooseneck	17.19	3.95	22.97	5.23	5.32	9.72	5.49	3.94
7	S. A. 182	16.50	3.75	22.60	5.31	5.67	8.70	4.97	3.47
8	Sugar Drip	15.66	3.90	24.90	5.34	5.40	7.41	3.81	3.37
9	Planter	16.01	6.75	42.16	7.58	7.54	5.73	3.07	2.50
10	Sourless	18.75	10.95	58.40	11.69	11.33	4.44	.91	3.39
11	Mazo Amber	15.94	7.90	49.56	8.60	8.34	4.50	2.45	1.87
12	S. A. 173	15.29	3.65	23.87	5.01	5.35	7.64	4.22	3.21
13	Early Folger (16154)	18.92	11.95	63.16	11.45	11.37	4.12	4.01	0
14	S. A. 186	13.40	3.55	26.49	4.65	4.44	6.97	4.19	2.58
15	Chinese Amber	14.10	4.15	29.43	5.06	5.11	6.10	3.55	2.38
16	S. A. 119–9	13.10	4.45	33.96	4.99	5.04	5.26	3.06	2.05
17	S. A. 169	16.19	9.10	56.81	9.25	9.19	4.52	3.05	1.34
18	Jones	14.69	8.95	60.92	8.74	8.40	3.99	2.93	.94
19	White African								
20	McLean								
21	Cowper								
22	Leoti	16.66	11.75	70.52	11.82	11.53	2.24	.44	1.73
23	Early Folger (9097)	15.86	11.90	75.04	11.85	11.64	.92	.19	.70
24	Albaugh								
25	S. A. 107	16.19	3.85	23.78	5.42	5.10	8.25	3.75	4.25
26	Colman	17.24	3.05	17.69	4.89	4.94	9.07	4.59	4.22
27	Minnesota Amber	12.95	1.65	12.74	2.81	3.10	7.93	4.61	3.09
28	S. A. 183	14.26	9.30	65.21	9.19	9.24	3.17	2.35	.74
29	Honey	13.26	6.50	49.01	6.79	7.14	4.69	3.36	1.20
30	S. A. 171	16.59	9.70	58.46	9.82	9.84	4.66	3.24	1.28
31	Kansas Orange								
32	Hodo	17.10	11.40	66.66	11.24	11.32	3.78	3.61	.07
33	Iceberg								
34	Silvertop								

[9] See pp. 683–684 of reference cited in footnote 5.

dextrose, and levulose correspondingly decrease; levulose disappears in the dead-ripe stage of four varieties.

Only one variety, No. 25, shows levulose in excess of dextrose through the three stages of maturity. Two varieties, Nos. 10 and 23, show levulose in excess of dextrose in the first and second stages of maturity. Variety No. 5 shows levulose in excess of dextrose in the first and third stages. Two varieties, 2 and 22, show levulose in excess of dextrose only in the milk stage of maturity. One variety, No. 4, shows dextrose in excess of levulose in the milk stage, a reversal in the dough-to-ripe stage and another reversal in the dead-ripe stage.

With the possible exception of variety 25 in which the excess of

TABLE 2.—*Analysis of juices of sorgo at the dough-to-ripe stage of maturity*

No.	Variety	Dissolved solids by Brix hydrometer	Sucrose by polarization	Apparent purity	Sucrose by— Clerget method	Sucrose by— Copper reduction	Reducing sugars as invert sugar	Dextrose	Levulose
		Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
1	S. A. 287	17.04	12.25	71.88	11.87	12.44	1.53	1 49	0
2	Red X	15.89	11 45	72.05	11.36	11.44	1.98	1.00	.93
3	Indiana Amber	18 84	9.95	52.81	10.69	10.53	5.67	3.19	2.30
4	S. A. 108	16.11	9.60	59.59	10.14	9.97	2.46	1.06	1.33
5	Straight Neck	18.01	12 95	71.90	12.93	13.35	2.18	2.00	.12
6	Gooseneck	15.34	6.05	39.43	6.35	6 26	6.85	3.53	3.12
7	S. A. 182	18 01	11.65	64.68	11.83	12.20	3.61	2.21	1.29
8	Sugar Drip	17.61	12.90	73.25	12.98	13.32	1.95	.96	.93
9	Planter	13.71	8.70	63.45	8.49	8 68	2 78	2.07	.64
10	Sourless	17.71	13.05	73.68	13.04	12.93	1 91	.55	1.30
11	Mazo Amber	16.01	9.75	60.81	9.97	9.56	2.39	1.68	.66
12	S. A. 173	16.19	10.60	65.47	10.66	10.53	3 10	1.70	1.31
13	Early Folger (16154)	21.74	16.85	77.50	16.97	17.22	.64	.51	.11
14	S. A. 186	15.46	7.15	46.24	7.62	7.81	5.71	3.39	2 15
15	Chinese Amber	15.01	8.10	53 96	8.49	8.53	3.11	1.72	1.29
16	S. A. 119-9	14.99	8.75	58.37	8.89	9.06	3.53	2.17	1.25
17	S. A. 169	17.94	11.40	63.54	11.53	11.73	4.33	2.95	1.26
18	Jones	15.91	10.80	67.88	10.20	10.40	3.59	2.31	1.18
19	White African	16.91	12.20	72.14	11.94	11.91	1 91	1.74	.10
20	McLean	16.31	12.05	73.88	12.05	12.21	1.18	.72	.43
21	Cowper								
22	Leoti								
23	Early Folger (9097)	16.54	11.65	70.43	11.63	11.56	2.08	1 00	1.02
24	Albaugh	14.15	5.25	37.10	5.79	5.56	6.26	3.64	2.44
25	S. A. 107	16.01	7.90	49.34	8.43	8.54	4.49	1.43	1.93
26	Colman								
27	Minnesota Amber								
28	S. A. 183	14.51	8.15	56.16	8.30	8.37	3.99	2.39	1.48
29	Honey	16.41	9.95	60.63	9.52	9.74	4.76	1.75	.28
30	S. A. 171	18.31	11.90	64.99	11.71	11.83	4.76	3.42	1.20
31	Kansas Orange								
32	Hodo	17.16	11.50	67.01	11.45	11.50	3.40	1.91	1.39
33	Iceberg								
34	Silvertop								

levulose over dextrose might be a varietal characteristic, no explanation appears to be warranted from the data for the occurrence of levulose in excess of dextrose in these other instances.

Table 4 is made up from average analytical data given in tables 1, 2, and 3 for the first 18 varieties. These varieties were selected because the analytical data were complete for all 3 stages of maturity and because none of them had widely varying growing seasons likely to introduce differences due to climatic conditions during growth.

A study of the data in table 4 permits some interesting deductions as to the type of dissolved solids and the synthesis of sucrose by the sorgo plant as it matures.

TABLE 3.—*Analysis of juices of sorgo at the dead-ripe stage of maturity*

No.	Variety	Dissolved solids by Brix hy-drometer	Sucrose by polar-ization	Appar-ent purity	Sucrose by—		Reduc-ing sugars as invert sugar	Dex-trose	Levu-lose
					Clerget method	Copper reduc-tion			
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	S. A 287	18 96	14.45	76.21	14.25	14 32	1 17	1.14	0
2	Red X	17.01	13.01	77 01	12 74	13.08	1.44	.95	.45
3	Indiana Amber	19.41	13 60	70 06	13 73	14.11	2.31	1.72	.52
4	S. A. 108	18.86	14 10	74.46	13.99	14.09	.93	.79	.11
5	Straight Neck	19.52	15.20	77.86	15.13	15.02	1.00	.19	.78
6	Gooseneck	14 51	6.35	43.76	5 91	6 07	6.23	4.52	1.53
7	S. A. 182	19.96	12.90	65.95	12.94	13.19	3.97	2.95	.91
8	Sugar Drip	18.86	14 65	77.67	14 67	14 30	1 03	.62	.39
9	Planter	16 50	11.85	71 81	11.77	11.47	2.32	1.60	.65
10	Sourless	19 46	15 15	77.85	15.20	15.33	1.19	.93	.23
11	Mazo Amber	17.31	11.60	67.01	11 64	11.58	1 96	1.05	.85
12	S. A. 173	19.64	13 95	71.02	14.03	14 02	2.22	1.43	.73
13	Early Folger (16154)	22 76	18 35	80.62	18.08	17.87	.56	.28	.25
14	S. A. 186	16.06	10.65	66.31	10.48	10.69	3.49	2 51	.78
15	Chinese Amber	17.54	10.75	61 28	11.41	11 60	2.72	1.60	1.04
16	S. A. 119-9	16.59	10.60	63.89	11.02	10.80	2.78	1.82	.88
17	S. A. 169	18.00	12.10	67 22	11 92	12.11	3.81	3.18	.52
18	Jones	15.30	10.35	67.64	9.87	9 66	3 40	2.54	.76
19	White African	18 26	13.90	76.12	13.62	13 83	1.39	1.35	0
20	McLean	16.86	12.80	75.81	11 83	12 11	1.27	1.23	0
21	Cowper	19 76	15.10	76.41	15.01	14.83	1.43	1.19	.20
22	Leoti	18.71	13.85	74.02	13.87	14.04	1.90	1.42	.44
23	Early Folger (9097)	18.11	13.70	75.64	13.54	13.65	62	.54	.06
24	Albaugh	15.01	8.25	54.96	8.32	8.67	4.13	3.79	.22
25	S. A. 107	18.64	12.05	64.64	12.41	12.76	2 80	1.14	1.58
26	Colman	18.91	12.70	67.16	13 09	13.44	2.22	1.29	.87
27	Minnesota Amber	13.14	7.60	57.83	7.58	7.87	3.14	2.47	.59
28	S. A. 183	15.90	11.15	70.12	11.01	10.87	2.69	1.73	.89
29	Honey	16.26	10.35	63.65	10.29	10.29	3.81	2.50	1.19
30	S. A. 171	18 06	12.45	68.93	12.07	12 60	3.40	3.31	0
31	Kansas Orange	17.06	12.45	72 97	12.11	12.26	2.39	1.89	.44
32	Hodo								
33	Iceberg	21.06	16 50	78.34	16.38	16.00	2 35	1.33	.94
34	Silvertop	19.00	13.95	73.42	13.55	13.33	3.55	2.65	.07

From the milk to dough-to-ripe stage of maturity the total dissolved solids expressed by degrees Brix increase from 16.03 percent to 16.75 percent, or 0.72 persent, and the total sugars increase from 13.38 percent to 13.95 percent, or 0.57 percent. The increase in total sugars in this stage is 79.16 percent of the total solids increase.

From the dough-to-ripe stage to the dead-ripe stage of maturity the total dissolved solids expressed by degrees Brix increase from 16.75 percent to 18.10 percent, or 1.35 percent, and the total sugars increase from 13.95 percent to 15.07 percent, or 1.12 percent. The

TABLE 4.—*Sorgo juices of varieties 1 to 18, inclusive—averages and calculated data*

Stage of maturity	Dis-solved solids in ex-pressed juice, by Brix hy-drom-eter	Sucrose in ex-pressed juice by Clerget method	Reduc-ing sugars as in-vert sugar	Total sugars— sucrose plus invert sugar		Total sugars calculated as invert sugar		Dex-trose	Levu-lose
				In ex-pressed juice	In per-cent of Brix solids	In ex-pressed juice	In per-cent of Brix solids		
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Milk stage	16.03	7.65	5.73	13.38	83.46	13.76	85.83	3.27	2.28
Dough-to-ripe stage	16.75	10.77	3.18	13.95	83.28	14.48	86.44	1.91	1.17
Dead-ripe stage	18.10	12.71	2.36	15.07	83.25	15.70	86.74	1.65	.63

increase in total sugars in this stage is 82.96 percent of the total
solids increase. The increases in total solids and total sugars between
the second and third stages of maturity are almost double those be-
tween the first and second stages; however, the solids acquired between
the second and third stages are higher in sugar than those acquired
between the first and second stages.

Table 4 shows that the total sugars as determined in the juice,
sucrose plus invert sugar, increase with maturity yet their percentage
of the Brix solids decrease slightly with maturity. When the equiv-
alent total sugars are calculated as invert sugar in terms of percent
Brix solids, we find the reverse is the case and the values increase with
maturity.

The decrease in percentage of reducing sugars as invert sugar from
the milk stage to the dough-to-ripe stage is 5.73 percent minus 3.18
percent (or 2.55 percent); this is not sufficient to account for a rise
in sucrose from 7.65 percent to 10.77 percent (or 3.12 percent) because
3.12 percent sucrose would require 3.12 percent times 1.05, or 3.28 per-
cent (instead of 2.55 percent) invert sugar for its synthesis.

Comparing the milk and dough-to-ripe stages of maturity, it is
noted that the increase in total sugars as invert sugar, from 13.76
percent to 14.48 percent (or 0.72 percent), added to the decrease in
reducing sugars as invert sugar, 2.55 percent, exactly equals the
amount of invert sugar required for the sucrose synthesis, 3.27 per-
cent.

When the dough-to-ripe and dead-ripe stages of maturity are simi-
larly compared, the same relations hold. That is, the reduction in
percentage of reducing sugars as invert sugar is not sufficient to
account for the sucrose synthesized by an amount equal to the increase
in total sugars, as invert sugar.

The average dextrose content of the juice exceeds the levulose con-
tent by nearly 1 percent in the three stages of maturity.

SUMMARY AND CONCLUSIONS

In a study of the sucrose, dextrose, and levulose content of 34
varieties of sorgo, it was found that:

(1) Sucrose content increased with maturity.

(2) In the milk stage of maturity the dextrose content exceeded
the sucrose content in 3 varieties (Nos. 3, 6, and 27).

(3) In all but 7 varieties (2, 4, 5, 10, 22, 23, and 25), the dextrose
content exceeded the levulose content for all stages of maturity
investigated.

(4) The levulose disappeared in 4 varieties in the dead-ripe stage
of maturity, in 1 variety in the dough-to-ripe stage, and in 1 variety
in the milk stage.

A comparison of the average analytical data, at all 3 stages of
maturity, for 18 varieties, having growing seasons which did not
vary too widely, permit the following conclusions:

(1) The increase in total sugars and total solids in the plant be-
tween the second and third stages of maturity are nearly double the
increase between the first and second stages. The total sugars, as
actually determined, are an increasing proportion of the increasing
total solids. The total sugars (sucrose plus invert sugar) as percent
of Brix solids as determined decrease slightly with maturity, while

their percentage in the juice increases. If the total sugars at each stage of maturity are calculated as equivalent invert sugar, so as to permit absolute comparison, they represent increasing percentages of the total solids in the juices from the later stages of maturity.

(2) The average quantity of sucrose synthesized in the juice of the plants in maturing exceeds the quantity that could have been formed from the average decreases in the invert sugar content of the juices. This excess was equivalent to the increase in total sugars as invert sugar.

(3) The average dextrose content of the juices in all three stages of maturity exceeds the levulose content by nearly 1 percent. Dextrose in excess of levulose may be expected to contribute to dextrose crystallization in sorgo juices where the total reducing sugars exceed the sucrose. This excess of dextrose over levulose is not so much greater than that found in invert sugar made by inversion of sucrose as to suggest commercial processing of the sorgo plant for crystalline dextrose.

REPRODUCTION AND MORTALITY OF CALIFORNIA RED SCALES RESISTANT AND NONRESISTANT TO HYDROCYANIC ACID GAS, AS AFFECTED BY TEMPERATURE [1]

By FRANCIS MUNGER [2]

Assistant entomologist, Division of Fruit Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

It was first pointed out by Quayle (*5*, *6*)[3] that in different areas the California red scale (*Aonidiella aurantii* (Mask.)), a major pest of citrus and other fruits, varies greatly in susceptibility to hydrocyanic acid gas. Quayle's findings have been corroborated by other workers. Dickson (*2*) and Yust et al. (*10*) have shown that resistance to hydrocyanic acid is an inherited sex-linked character. Yust et al. (*11*) and Lindgren and Dickson (*3*) have also shown that repeated fumigations of different stocks of scales may gradually increase their resistance to hydrocyanic acid. Since most field populations of red scale are probably mixtures of resistant and nonresistant scales, the selective action of repeated fumigations might tend to make the insect more difficult to control, unless biological factors operate to favor the nonresistant scale. An investigation of the comparative biology of the two strains was therefore undertaken at Whittier, Calif. The present paper reports the results of studies on reproduction and mortality of the two strains under different temperatures.

METHODS

Two stocks of scales were originally collected in the field by F. S. Stickney in 1935, the nonresistant strain from Escondido and the resistant strain from Corona, Calif. They were reared in isolated compartments at Whittier from 1935 to 1946, inclusive, with no substantial change in their relative resistance to hydrocyanic acid. In a number of parallel tests with hydrocyanic acid gas on different generations, the mortality of the second-molt [4] scales averaged 99.3 percent for the nonresistant and 32.5 percent for the resistant strain (*10*). Although the relative susceptibility of the two strains varied considerably on different dates, no consistant increase or decrease was

---

[1] Received for publication June 25, 1947.
[2] The author is indebted to A. W. Cressman for advice and suggestions during the course of these studies.
[3] Italic numbers in parentheses refer to Literature Cited, p. 162.
[4] Definitions of stages throughout this paper follow Yust et al. (*8*).

Journal of Agricultural Research,
Washington, D. C.

Vol. 76, Nos. 7, 8
April 1, 15, 1948
Key No. K-343

(153)

evident. These stocks were used in the first part of the work, but beginning in December 1942, most of the resistant scales were taken from a part of the resistant stock that had been given 14 fumigations in 15 generations (*11*).

The methods of rearing the scales were similar to those described by Yust and Munger (*9*), except that in the mortality studies a half-lemon technique was used, to eliminate variability due to individual fruit differences. A sticky banding material, smeared on a paper ribbon or on a piece of twine, was fastened around the middle of each lemon. Both strains of the scale could then be reared on the same fruit. This method could not be used in the reproduction studies in which records on settling were desired, but fruits were carefully selected for uniformity.

REPRODUCTION

AT CONSTANT TEMPERATURE

The first group of scales was subjected to a constant temperature of 77° F. and 65 percent relative humidity.[5] Individual records on crawler production were kept during the entire life of the females. The individual scales were confined within gelatin capsules, as described by Yust (*7*), but methyl alcohol, instead of ethyl alcohol, was used in the sealing paste. Thirty-two mature scales of each strain were thus confined, but decay of a number of lemons permitted only 22 resistant and 19 nonresistant scales to complete their reproductive period. Inspections were made daily. As shown in the first line of table 1, the resistant scales produced an average of 305.7 live crawlers per female, and the nonresistant scales an average of 326. The difference was not statistically significant.

TABLE 1.—*Reproduction of California red scales under different temperature conditions*

Rearing condition	Duration of experiment	Reproducing females		Scales produced per female	
		Resist-ant	Nonre-sistant	Resist-ant	Nonre-sistant
		Num-ber	*Num-ber*	*Num-ber*	*Num-ber*
77° F. constant and 65 percent relative humidity.	Entire reproductive period of scales.	22	19	305.7	326.0
Laboratory, high fluctuating temperatures.	7 weeks	95	95	64.5	64.1
Winter lath house	Nov. 6, 1942–Apr. 16, 1943	100	100	135.5	148.5
Summer lath house	July 5–Sept. 9, 1943	60	60	113.1	217.4
Do	July 10–Aug. 23, 1943	60	60	79.2	146.9

AT HIGH FLUCTUATING TEMPERATURES

A second group of scales was subjected to controlled fluctuating temperatures which duplicated thermograph records from Corona, Calif., for the 4-week period July 15 to August 11, 1933. These temperatures were representative of hot, interior California conditions and were reproduced in the laboratory for 7 weeks by means of patterns

[5] The constant-temperature studies were made by B. M. Broadbent.

for the variable thermostat (Munger *4*), first using the 4-week record and then repeating records for weeks 1, 3, and 4. Week 2 was omitted from the second period because it was a week of abnormally high temperatures, 4 days being well above 100° F. The maximum, minimum, and mean daily temperatures for this period are shown in figure 1. Moisture in the chamber was maintained by means of an
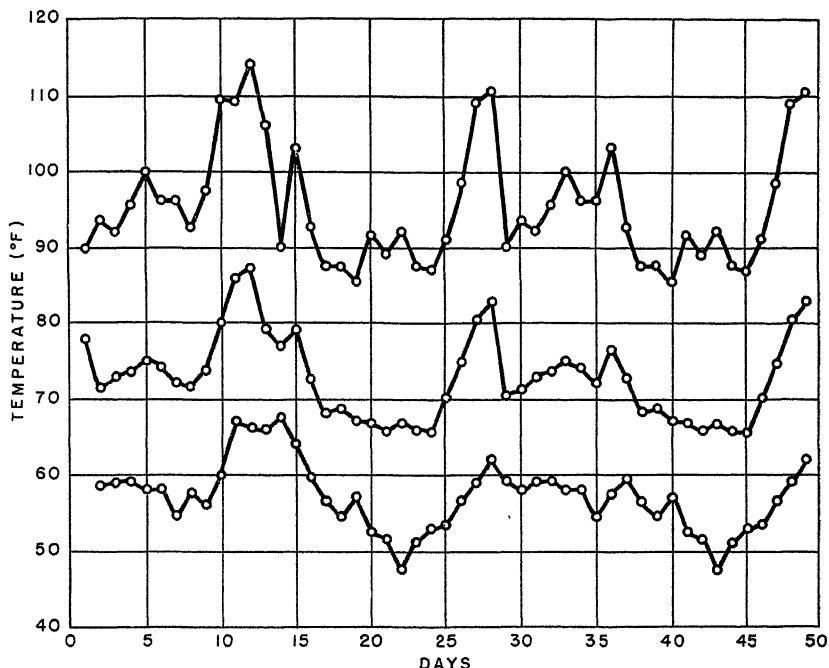
FIGURE 1.—Maximum, mean, and minimum daily temperatures of the 7-week program in which Corona, Calif., temperatures were reproduced in the laboratory.

open pan filled with water, over which air was continuously forced by means of a blower.

On November 12, 1943, 50 lemons were lightly infested, 25 with resistant and 25 with nonresistant scales. The scales were held at 77° F. until the beginning of the 7-week fluctuating-temperature program. As the scales approached the reproducing stage all but 5 mature females were removed from the lemons. On December 29 the experiment was begun. At that time a few crawlers could be seen under the covers of some of the females, but no whitecap stages were observed on the lemons.

The young scales were removed and records were made at weekly intervals. At the end of 7 weeks, 19 of the lemons in each group were in good condition. The results of this experiment, based on the counts from the 38 good lemons, are shown in the second line of table 1. On these lemons 74 resistant and 63 nonresistant scales remained alive. At the end of the first to sixth counts, inclusive, the totals of living resistant and nonresistant reproducing scales were 95 and 95, 89 and 89, 83 and 79, 80 and 73, 79 and 69, and 77 and 69, respec-

tively. The difference between the reproduction of the 2 strains, 0.4 scale per female (table 1), was not statistically significant.

IN THE WINTER LATH HOUSE

Three groups of scales were studied under various lath-house conditions. On September 16, 1942, 2 groups of 33 lemons each were infested lightly, one with the resistant and the other with the non-resistant strain of scale. The scales were held at 77° F. until reproduction began, and then all but 5 mature female scales and the few young that had been produced were removed from each lemon. The scales were placed in the lath house on November 6, 1942. The young scales were removed and counted at intervals of 11 to 22 days between November 19, 1942, and April 16, 1943. During this period the temperature ranged from 37° to 90°. Each of these extremes occurred but once, and on only 4 days did the temperature fall below 40°.

At the time of the April 16 count, 20 lemons of each group were still in good condition, and on them 86 resistant and 88 nonresistant scales were still alive. During the reproduction period measured (table 1) the nonresistant scales produced 13 more young per scale than did the resistant scales, but this difference was not statistically significant.

IN THE SUMMER LATH HOUSE

The third and fourth groups of scales, prior to being placed in the lath house, had been subjected to a 24-day period of fluctuating temperatures, with slowly decreasing minimum temperatures designed to harden the scales to withstand low temperatures in the first- and second-molt stages. From the twentieth to the twenty-second day of the 24-day period, while the scales were mostly in the molting stages, they were subjected to a minimum temperature of 29° F. on three successive nights. The scales that encountered the low temperatures in the second-molt stage were in the lath house from July 5 to September 9, 1943. The ones that encountered the low temperatures in the first-molt stage were in the lath house from July 10 to August 23, 1943. In both these summer-lath-house tests the nonresistant scales produced considerably more young than the resistant scales. This greater production, however, was linked with a higher survival of the nonresistant females.

Such a difference in productivity would be of considerable importance if it were consistent, and therefore a number of similar experiments were made. Since the mortality of the reproducing females, rather than the production per living female, was shown to be the important factor in determining the number of young, future data were limited to the mortality during the reproducing period, thus permitting the use of a much larger number of scales. The scales were exposed to low temperatures, as in previous tests, and were then held at 77° F. until reproduction began, after which they were placed in the lath house. Results of additional tests are given in table 2.

Only 1 of the 4 groups (first molt, observed from July 31 to Sept. 11, 1944) showed any great difference in mortality. This group was

TABLE 2.—*Mortality of reproducing California red scales in the lath house at Whittier, Calif.*

Stage of scale exposed to low temperature	Scales of each strain	Reproductive period observed	Mortality of scales	
			Resist-ant	Nonre-sistant
	Number		*Percent*	*Percent*
First molt	600	Aug. 6–Oct. 19, 1943	58 0	60.0
Second molt	1,200	Aug. 6–Sept 20, 1943	9.6	4.8
Do	1,200	July 22–Aug. 25, 1944	5.9	5.8
First molt	600	July 31–Sept. 11, 1944	52.8	19.2

exposed to rather high temperatures on 2 days (maxima, 105° and 103° F.) shortly before the mortality counts were made, in contrast to the other groups, which had encountered no high temperatures. Since it was possible that the combination of low temperatures in the molting stages and high temperatures during the reproductive period had caused an abnormal mortality of the resistant scales, tests were made, as follows: After the scales on 12 lemons had been exposed to low temperatures in the first-molt stage, they were held at 77° until reproduction began, and then 6 of the lemons were subjected to alternate weekly hot and cool temperatures from November 23 to December 27, 1944. The daily maximum in the hot week ranged from 90° to 105°, and the maximum in the cool week was 80°. The other 6 lemons were kept at 77°. Scales on a second lot of 12 lemons were not exposed to low temperatures in the first molt, but were reared at 77° until reproduction began, when they were divided, as was the first lot. The highest mortality in any of these groups was 23 percent, and there was no difference between the 2 strains. Tests with a large number of female scales have therefore failed to show any consistent difference in mortality which would lead to differences in the number of young produced.

Although the factors responsible for the three instances of differences in the mortality of the reproductive stage after earlier conditioning to low temperatures have not been isolated, it does not seem likely that the occasional occurrence of these differences indicates any important biological advantage for the nonresistant strain. The conditioning of the first- and second-molt stages required a fall in temperature much more gradual than is common in nature. Very few of the first- and second-molt stages survive winter temperatures in the field.

MORTALITY

Records on mortality were obtained in a lath house on the laboratory grounds at Whittier, Calif., in different seasons and also under various controlled fluctuating temperatures.

IN THE LATH HOUSE

Scales were reared at 77° F. until they reached the desired stages. Equal numbers of scales of each strain were then marked on each lemon and the fruit was placed in a lath house to determine the effect of outdoor conditions on mortality. The results are summarized in table 3.

Table 3.—*Mortality of California red scales in the lath house, Whittier, Calif* [1]

Period in lath house	Stage of scale placed in lath house	Scales of each strain	Mortality of scales	
			Resistant	Nonresistant
		Number	*Percent*	*Percent*
Nov. 21, 1941–Mar. 5, 1942	Whitecap	300	97.0	96.0
Feb. 17–Apr. 21, 1942	do	1,200	75.2	72.7
Nov. 18, 1942–Apr. 16, 1943	do	600	95.3	92.2
Mar. 2–18–June 3–15, 1943	do	2,160	75.6	62.3
Apr. 3–June 18, 1943	do	600	58.0	50.8
June 26–27–Aug. 24–28, 1943	do	2,400	43.8	61.9
Nov. 12, 1942–Apr 2, 1943	First molt	300	91.7	84.3
Dec. 5, 1941–Mar. 27, 1942	Second stage	90	78.8	81.1
Nov. 28, 1942–Apr 14, 1943	do	600	63.5	77.8
Dec. 5, 1941–Mar. 27, 1942	Second molt	90	73.3	78.8
Nov. 25, 1942–Apr. 13, 1943	do	360	81.7	84.1
Dec. 19, 1941–Mar 30, 1942	Gray adult	30	36.6	23.0
Dec. 7, 1942–Apr. 17, 1943	do	300	65.7	63.0
Jan. 2, 1942–Mar 30, 1942	Mature	120	44.2	10.0
Nov. 10–23, 1942–Mar. 3–Apr. 1, 1943	do	1,800	14.8	14.7

[1] Some groups include scales that settled and were put in the lath house on different days.

There seemed to be no consistent differences in mortality between the two strains. In four groups the differences in mortality were statistically significant when each group was analyzed separately. In two groups (March 2–18—June 3–15, 1943, and November 12, 1942—April 2, 1943) the resistant strain had the greater mortality; in two (June 26–27—August 24–28, 1943, and November 28, 1942—April 14, 1943) the mortality was greater in the nonresistant strain.

Whitecap scales kept in the lath house through the winter months suffered very high mortality—96.5 percent in 1941–42 and 93.8 percent in 1942–43. Most of the surviving scales were mature when the final mortality counts were taken, but records were made of the stages in which death occurred. The molting stages were the most critical. During the months of March to June, inclusive, only whitecaps were put in the lath house, but the surviving scales had reached the mature stage by the time final records were made. The mortality of such groups as are recorded in table 3, therefore, is a summation of scales dying in all stages.

The lowest temperatures for the winter of 1941–42 were in December, January, February, and March, with minima of 37°, 35°, 33°, and 38° F., respectively. In the same months for the winter of 1942–43 they were 38°, 39°, 37°, and 44°, respectively.

AT HIGH FLUCTUATING TEMPERATURES

In tests with a variable-temperature thermostat, scales were subjected to artificially fluctuating temperatures and to selected summer- and winter-temperature patterns taken from thermograph records at Corona, Calif. The Corona records were selected as representative of interior conditions in a region where the red scale is a severe pest. Earlier studies by Munger [6] had shown that the body temperature of scales in the shade corresponds closely to the air temperature, whereas that of scales exposed to the sun under California conditions may be

[6] MUNGER, F. BODY TEMPERATURE MEASUREMENTS OF THE CALIFORNIA RED SCALE. Unpublished manuscript.

more than 21° above the air temperature. Some of the present tests were based on scale temperatures in the sun, but most of them were based on shade temperatures, since the bulk of the normal scale population is in the shade. Prior and subsequent to the fluctuating-temperature tests, the scales were reared at 77° F. Mortality counts were made about 3 weeks after the scales were removed from the fluctuating-temperature cabinet.

A high-temperature program consisted of a reproduction of the Corona thermograph record for the week ending July 28, 1933. This record was chosen primarily because the day temperatures were high. The maximum daily temperatures for this week were as follows: 92.5°, 97°, 99°, 99°, 113°, 106°, and 90° F. The lowest temperature was 56° on the first night. Scales of all stages (see table 4) were used in

TABLE 4.—*Mortality of California red scales in a fluctuating-temperature cabinet in which the high temperatures of Corona, Calif., were reproduced*

| Stage of scale | Scales of each strain | Mortality of scales | | Stage of scale | Scales of each strain | Mortality of scales | |
		Resist-ant	Nonre-sistant			Resist-ant	Nonre-sistant
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*
First stage	600	21 3	19 0	Second molt	1,200	27.4	35 2
First molt	900	34. 2	32 7	Gray	360	2.8	3.3
Second stage	240	1 3	8	Mature	1,570	9.9	9.6

this week-long program. In the first and second molts and in the mature scales, results with several groups of scales have been averaged. There was no consistent difference in mortality between the strains. The molting stages and the first stage suffered higher mortality than the second, the gray, or the mature stages. A group of 4,000 scales, reared continuously at 77° from the time of settling to the beginning of reproduction, suffered a total mortality of 28.6 percent, with no differences between the strains.

Since the temperature of scales and of fruit surfaces in the sun may rise well above air temperature, an attempt was made to measure the effect of high temperatures, such as might be encountered occasionally by exposed scales. The temperature pattern followed was based in part on thermograph records covering a period of hot weather from September 6 to 9, 1937. During this period the daily temperature ranges were 52° to 98°, 59° to 106°, and 62.5° to 108.5° F., with a final maximum of 110°. The maximum temperatures were corrected by adding the differences between fruit and air temperatures, found by means of thermocouples. The highest differential between air and fruit temperature was 15.2°, which was found when the air temperature registered 106.3°. The complete sequence of minimum and maximum temperatures used in tests of scales of all stages consisted of the following: 52°–109°, 58°–115°, 59°–119.5°, 62.5°–121.5°, 59°–119.5°, 58°–115°, and 52°–109°. The complete test killed all scales and burned the lemons. In tests in which only the first 3 and first 4 days (except for the fourth minimum) were used, some of the scales survived (see table 5).

TABLE 5.—*Mortality of California red scales exposed to fluctuating high temperatures based on temperatures of fruits in the sun when air temperatures are high*

Stage of scale	Mortality of scales exposed first 3 days		Mortality of scales exposed first 4 days	
	Resistant	Nonresistant	Resistant	Nonresistant
	Percent	*Percent*	*Percent*	*Percent*
Whitecap	48.5	47.8	96.7	93.8
First molt	92 7	92 7	99.3	98.0
Second stage	45 6	45.6	92.0	92.0
Second molt	99.2	100.0	100.0	100 0
Gray stage	90.7	92.0	98.0	98 7
Mature	100.0	100.0	100.0	100.0

Although there was practically no difference in mortality between the strains, the whitecap and second stages were the least susceptible to high temperatures.

AT LOW FLUCTUATING TEMPERATURES

Scales were next exposed to lower temperatures, according to a program based in part on the thermograph record of the last week in October 1935 at Corona. It ranged from 55°–94°, 45°–90°, 50°–82°, 39°–66°, 35°–64°, and 37°–70° F., and differed from the recorded temperatures mainly in that the minimum temperatures of the last 5 days were 2°, 3°, 2°, 6°, and 4° higher than the field temperatures. Whitecap, second-molt, and gray stages were used simultaneously in the test. Only 6.7 percent of the gray-stage scales were killed, as compared with 94.6 percent of the second-molt ones. There was no difference between the strains in these two stages. The whitecap resistant and nonresistant scales suffered mortalities of 26.2 and 19.7 percent, respectively.

Further tests were made which provided for a more abrupt drop in temperature near the midpoint. The minimum and maximum daily temperatures used were as follows: 57°–93°, 56°–93°, 55°–93°, 30°–90.5°, 30°–61°, 35°–67.5°, and 53°–62° F. Mature scales used in these tests showed an average mortality of only 11 percent, while second-molt scales came within a fraction of 1 percent of being completely destroyed. In another experiment in which an 11-day program was used, with minimum temperatures of 57°, 56°, 55°, 41°, 57°, 56°, 55°, 31°, 31°, 31°, and 31°, first-molt scales suffered an average mortality of 95.5 percent, while mature scales suffered an average mortality of only 14 percent, with no difference between the strains.

The results suggested that the molting stages might be conditioned to withstand low temperatures. To test this possibility two programs were used. In the first, the minimum and maximum temperatures were as follows: 60°–80°, 55°–75°, 50°–73°, 45°–70°, 43°–73°, 41.5°–72°, 42°–57.5°, 34.5°–55°, 34.5°–58°, 34°–59°, 34°–60.5°, 41°–56.5°, 41.5°–71°, 42°–57.5°, 34.5°–55°, 34.5°–58°, 34°–59°, 34°–60.5°, 41°–56.5°, and 41.5°–72° F. Marked scales were in the first- and second-molt stages from the fifteenth to the eighteenth days of the program, when the temperatures reached a minimum of 34°. The mortality of these scales was low, the highest being 10.3 percent in the

resistant scales in the first molt. In both strains the mortality of the second-molt scales was less than 2 percent.

The second low-temperature conditioning program carried out included lower minimum temperatures than the first, and consisted of 24 days in which the daily minimum and maximum temperatures were as follows: 60°–80°, 55°–75°, 50°–73°, 45°–70°, 43°–73°, 41.5°–72°, 42°–57.5°, 34.5°–55°, 34.5°–58°, 34°–59°, 34°–60.5°, 41°–56.5°, 55°–75°, 50°–73°, 45°–70°, 43°–73°, 41.5°–72°, 42°–57.5°,34.5°–55°, 29°–61.5°, 29°–61.5°, 29°–61.5°, 40°–65°, and 50°–70° F.

By the time the 29° F. minimum temperatures occurred, the first- and second-stage scales in the cabinet had progressed to the first- and second-molt stages. The results of this experiment (table 6)

TABLE 6.—*Relation between development of molting scales and their susceptibility to low temperature*

Stage and development	Mortality of scales	
	Resistant	Nonresistant
	Percent	*Percent*
Late first molt	17.0	18.0
Early first molt	11 7	8.7
Late second molt	42.8	42.2
Early second molt	5 0	1.7

showed clearly that the conditioning period had served to protect many of the scales from being killed by the low temperatures, which in previous tests had produced a high mortality. It was also apparent that the stage of development of the molting scales was related to their susceptibility to low temperatures.

DISCUSSION

Conditions to which resistant and nonresistant strains of the California red scale were subjected in these experiments ranged from low temperatures, which killed nearly all the molting stages, to temperatures high enough to injure the host fruit. No consistent differences between the reactions of the two strains were noted. At certain times scales of one strain were more tolerant of adverse temperatures than those of the other strain, but such differences might be reversed in a later generation.

The tests have furnished considerable information on the conditions that affect mortality of the California red scale. The range of higher temperatures up to 113° F. caused mortality averaging as high as 33.4 and 31.3 percent in the first- and second-molt stages, respectively. Eight hundred scales of both strains, reared at a constant temperature of 77° and marked in the second-molt stage, suffered an average mortality of only 2.3 percent up to the time when reproduction began. A temperature of 121.5° caused complete mortality of some stages, but injury to the fruit may have been a contributing factor. Under field conditions only a small proportion of the scales would ever be exposed to such temperatures. The effect of winter temperatures on

the molting stages is more important, and these experiments showed that a rapid drop in temperature may kill most of the first- and second-molt stages, even though the minimum temperatures remain above freezing. Bliss et al. (*1*, *p. 1228*) reported that, in their experiments at Whittier, "Practically none of the new scales settling later than October managed to reach the adult stage and reproduce." In the tests here reported, only 3.5 and 6.2 percent of the whitecaps set out in a lath house in November survived the winter. Minimum temperatures at the laboratory were somewhat higher than those recorded in many nearby citrus groves on lower ground. It is probable, therefore, that in the field very few of the scales produced late in the fall survive the winter.

SUMMARY

Reproduction and mortality of two strains of the California red scale (*Aonidiella aurantii* (Mask.) one resistant and the other non-resistant to hydrocyanic acid gas, were studied under various temperature conditions.

There was no consistent evidence that reproductive capacity or mortality was linked with susceptibility to hydrocyanic acid fumigation.

High fluctuating temperatures up to a maximum of 113° F. increased the mortality of the second-molt stage by an average of 29 percent over that observed at 77°. In the laboratory a temperature of 121.5° caused complete mortality of some stages, but in nature relatively few scales are exposed to so extreme a temperature.

The molting stages were the ones most susceptible to low temperatures. A sharp drop to 29° F. caused almost complete mortality of scales in the first- and second-molt stages. Mortality was lowest when temperatures were gradually decreased to the same minimum.

The evidence indicates that few of the scales produced late in the fall survive the winter.

LITERATURE CITED

(1) BLISS, C. I., BROADBENT, B. M., and WATSON, S. A.
    1931. THE LIFE HISTORY OF THE CALIFORNIA RED SCALE CHRYSOMPHALUS AURANTII MASKELL: PROGRESS REPORT. Jour. Econ. Ent. 24: 1222- 1229, illus.
(2) DICKSON, R. C.
    1941. INHERITANCE OF RESISTANCE TO HYDROCYANIC ACID FUMIGATION IN THE CALIFORNIA RED SCALE. Hilgardia 13: 515- 522, illus.
(3) LINDGREN, D. L., and DICKSON, R. C.
    1945. REPEATED FUMIGATION WITH HCN AND THE DEVELOPMENT OF RE-SISTANCE IN THE CALIFORNIA RED SCALE. Jour. Econ. Ent. 38: 296–299.
(4) MUNGER, F.
    1944. AN ADAPTATION OF A THERMOGRAPH TO REGULATE VARIABLE TEM-PERATURE. Jour. Econ. Ent. 37: 554- 555, illus.
(5) QUAYLE, H. J.
    1915. THE CONTROL OF CITRUS INSECTS. Calif. Agr. Expt. Sta. Cir. 129, 36 pp., illus.
(6) ———
    1916. ARE SCALES BECOMING RESISTANT TO FUMIGATION? Calif. Univ. Jour. Agr. 3: 333–334, 358.

(7) YUST, H. R.
    1943. PRODUCTIVITY OF THE CALIFORNIA RED SCALE ON LEMON FRUITS.
        Jour. Econ. Ent. 36: 868–872, illus.
(8) ———, BUSBEY, R. L., and HOWARD, L. B.
    1942. LABORATORY FUMIGATIONS OF THE CALIFORNIA RED SCALE WITH
        METHYL BROMIDE, ALONE AND WITH HYDROCYANIC ACID. Jour.
        Econ. Ent. 35: 521–524.
(9) ———, and MUNGER, F.
    1943. CALIFORNIA RED SCALE. *In* Laboratory Procedures in Studies of
        the Chemical Control of Insects. Amer. Assoc. Adv. Sci. Pub.
        20: 34–35, illus.
(10) ———, NELSON, H. D., and BUSBEY, R. L.
    1943. COMPARATIVE SUSCEPTIBILITY OF TWO STRAINS OF CALIFORNIA RED
        SCALE TO HCN, WITH SPECIAL REFERENCE TO THE INHERITANCE
        OF RESISTANCE. Jour. Econ. Ent. 36: 744–749, illus.
(11) ———, NELSON, H. D., and BUSBEY, R. L.
    1943. THE INFLUENCE OF REPEATED FUMIGATION WITH HCN ON THE SUS-
        CEPTIBILITY OF THE CALIFORNIA RED SCALE. Jour. Econ. Ent.
        36: 872–874.

THE PRODUCTION OF ALATE FORMS OF MYZUS PERSICAE ON BRASSICA CAMPESTRIS IN THE GREENHOUSE [1]

By W. A. SHANDS, *senior entomologist, Bureau of Entomology and Plant Quarantine,* and G. W. SIMPSON, *associate entomologist, Maine Agricultural Experiment station, and collaborator, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

The green peach aphid (*Myzus persicae* (Sulz.)) reduces the yield of potatoes both through direct feeding injury and through the transmission of potato virus diseases. In Maine winged aphids are largely responsible not only for the initial infestation of potato plants early in the season but also for the wide dispersal of the species and for the continued reinfestation of potato plants throughout much of the summer.

Shands, Bronson, and Simpson (4) [3] reported that wild rutabaga (*Brassica campestris* L.) growing in the northeastern part of Maine was an important secondary host on which relatively large numbers of winged, or alate, forms of this aphid developed. In 1942 the present experiment was conducted at Presque Isle to secure information bearing upon the probable importance of this common weed as a host for alate forms of the green peach aphid. The two experimental variables were stage of maturity at which the plants were infested and size of the population with which they were infested.

METHODS OF EXPERIMENTATION

Six-inch flowerpots were used for all plants. Planting dates were so spaced that, when the experiment began, plants in four stages of growth were available, namely, very young, young, early-flower, and mature. Each pot had a single plant when the experiment began except the pots that contained the very young plants, which had four. Soon after colonization these were thinned to a single plant per pot.

[3] Italic numbers in parentheses refer to Literature Cited, p. 173.
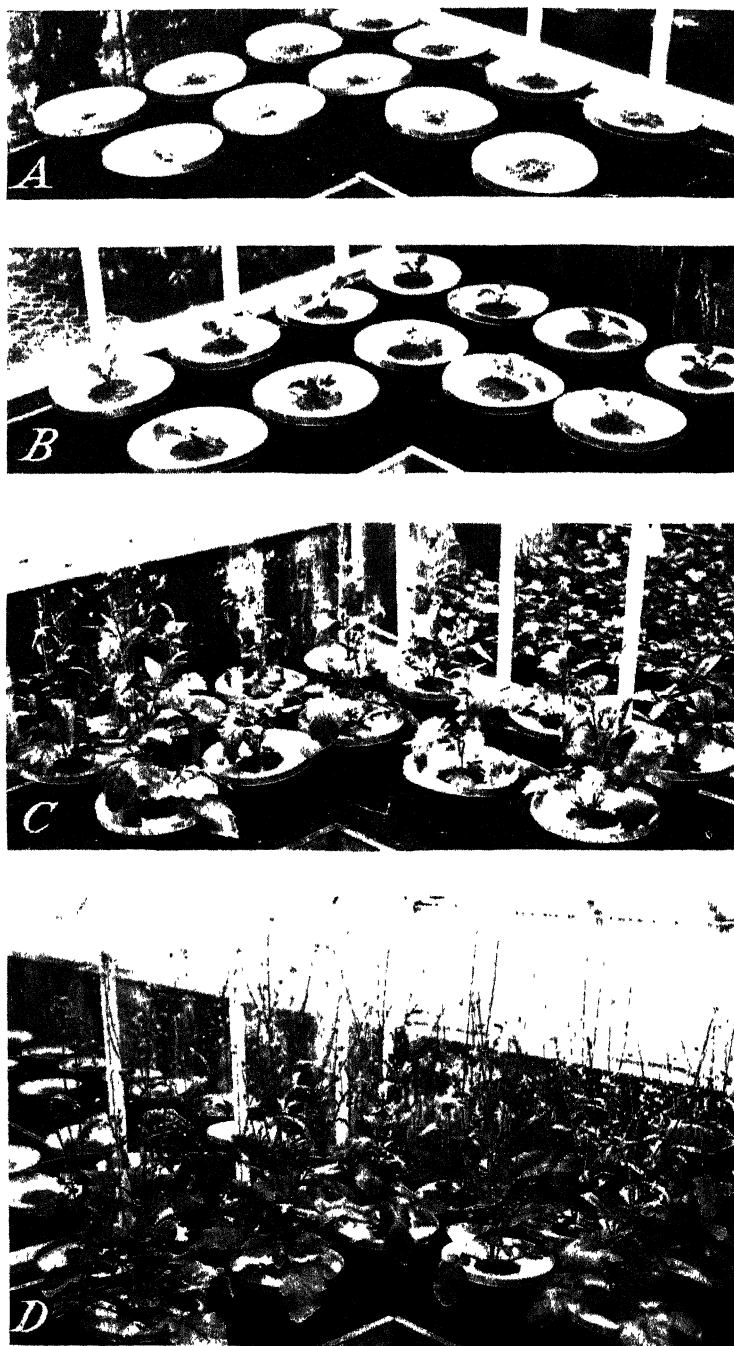
FIGURE 1.—Plants of *Brassica campestris* when the experiment began: *A*. Very young plants (two-leaf stage) ; *B*, young plants; *C*, early-flower plants ; and *D*, mature plants.

Figure 1 shows typical plants in each stage of growth at the beginning of the experiment. The very young plants were in the seedling stage, and the mature plants, although still flowering, had a few seed pods which were practically mature but still green.

Three sizes of aphid population were employed for infesting plants of each stage of maturity; namely, 1, 10, and 100 apterous individuals per plant. There were thus 12 treatments with 8 replications each, arranged in the form of a randomized block. The replicates were divided equally between two similar, adjoining rooms in the greenhouse.

The mature, apterous aphids that were used to infest the plants had been reared in the greenhouse, principally on wild rutabaga plants. All the plants to be used in the experiment were fumigated with a

FIGURE 2.—Caged plants of *Brassica campestris*.

nicotine smoke on May 26, and between May 27 and 29 aphids were transferred to the test plants. Selection of the mature apterous individuals was made with the aid of a binocular microscope just before the plant was to be colonized, and immediately after colonization the plant was covered with a cage. Except for short periods of examination for removal of alate forms, each plant remained under its cage until death.

A circular metal pan (fig. 1) with a 4¾-inch opening in the center rested on the top of each pot and held the cage securely over the plant. The cylindrical cage, approximately 13 inches in diameter and 19 inches tall, was covered with tobacco-plant-bed cloth having 32 by 28 threads per square inch. Figure 2 is a general view of some of the caged plants in two greenhouse rooms.

Throughout the experiment, except for 2 days of unusually hot weather when the plants were watered twice a day, the daily watering consisted of 130 to 135 cc. of ordinary tap water poured at one time into the saucer of each pot.

On June 12, when appreciable numbers of alate forms were first seen in the cages, each plant was carefully examined for alate forms of the aphid, and these examinations continued at intervals of 3 to 6 days until the plants died. All specimens found were removed by suction and preserved for later counting. The facilities available did not permit a determination of the number of apterous individuals per plant at any time after colonization.

FIGURE 3.—Dead plants of *Brassica campestris* at the close of the study. From left to right, stages of growth are very young, young, early-flower, and mature.

EXPERIMENTAL RESULTS

By August 15 most of the plants were dead, but a few lived until the end of the month. Table 1 shows the total number of alate forms recovered from each plant and the average per plant of each subclass. A study of the data by analysis of variance disclosed that the number of alate forms recovered from very young plants was significantly smaller than the number recovered from plants in other stages of growth, irrespective of the size of the initial population. Very young plants were too small to support the initial populations employed; consequently many of the little plants soon died. Others, while not actually killed by the aphids, were soon in such poor condition that the population was greatly reduced. In such instances the plants recovered before the population increased to any extent, but populations never attained a high level and relatively few alate forms were produced. Figure 3 shows the typical appearance of dead plants at the conclusion of the experiment.

TABLE 1.—*Alate forms of Myzus persicae recovered from caged plants of wild rutabaga growing in the greenhouse when size of initial population and stage of plant growth were variables*

Initial number of apterous individuals per plant	Plant No.	Number of alate individuals recovered from—			
		Very young plants	Young plants	Early-flower plants	Mature plants
1	1	114	2,173	1,972	2,118
	2	413	2,804	2,260	3,088
	3	49	3,250	1,201	3,483
	4	496	1,727	3,797	3,769
	5	1,658	278	1,993	2,363
	6	105	3,415	1,642	703
	7	1,345	2,832	1,231	2,085
	8	93	3,660	2,184	1,253
Average		534 1	2,517 4	2,035 0	2,357.8
10	1	182	3,445	2,340	1,222
	2	232	5,363	3,634	2,944
	3	106	2,262	2,526	1,393
	4	80	3,194	3,766	611
	5	256	3,485	2,162	2,141
	6	99	6,174	2,056	2,327
	7	236	1,783	1,704	2,298
	8	165	2,101	2,070	2,048
Average		169.5	3,475.9	2,532.2	1,873.0
100	1	657	4,486	3,051	1,843
	2	24	583	4,037	3,453
	3	169	1,199	1,138	1,679
	4	26	299	3,719	754
	5	65	2,335	3,163	1,691
	6	16	1,910	1,573	1,674
	7	0	4,250	5,566	3,197
	8	26	1,137	1,961	2,769
Average		122.9	2,024.9	3,026.0	2,132.5

Differences between means of subclasses required for significance ($P=0.05$) 1018 and ($P=0.01$) 1355.

Among the other 3 age groups infested with 1 aphid per plant the differences in number of alate forms recovered were not significant (table 1). However, when 10 aphids per plant were employed, the number of alate forms recovered from young plants was almost significantly greater than that recovered from plants in the early-flower stage, and the increase over that from mature plants was highly significant. With an initial population of 100 apterous individuals per plant, the number of alate individuals recovered from early-flower plants approached significance over that from young plants.

When plants of the same stage of growth at colonization but differing in the size of the initial population are considered (table 1), it is apparent that for young plants the number of alate forms recovered from an initial population of 10 apterous individuals per plant was almost significantly greater than that from a colony of 1 per plant, and the increase over that from a colony of 100 per plant was highly significant. For early-flower plants the number of alate forms from a colony of 100 per plant was almost significantly greater than that from a colony of 1 per plant. Differences were not significant for mature plants with different sizes of initial population.

A study of the data showing the number of alate forms recovered at each period of observation suggested that differences might have existed among subclasses in the time and rate at which winged forms

matured. Figure 4 shows the average number of alate forms per
plant per day recovered from young, early-flower, and mature plants
for each size of initial population. The data for constructing the
graphs were computed by dividing the average number of alate forms
per plant of each subclass recovered on a given date by the number of
days since the previous observation. An arbitrary time of 4½ days
was used in deriving the first point on all curves, since it seemed un-
likely that many alate forms could have matured under the existing
conditions in less than 10 days after colonization.

Figure 4 shows that, in general, production of alate forms began
first on plants having the largest initial population, followed in order
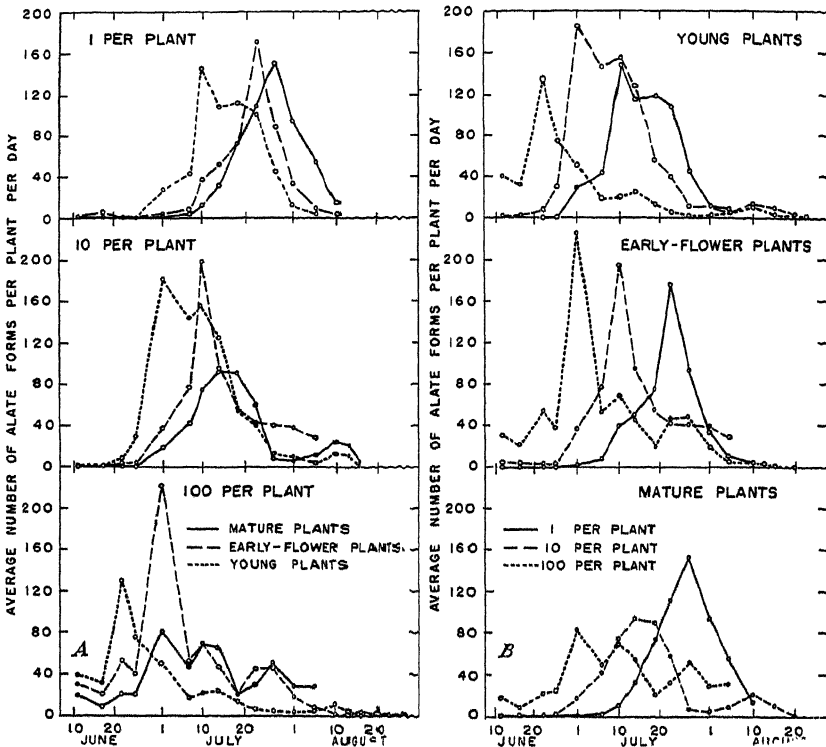
FIGURE 4.—Average number of alate forms of *Myzus persicae* recovered per plant
per day from young, early-flower, and mature plants when initial infestation
was, respectively, 1, 10, and 100 apterous individuals per plant. *A*, Data
grouped according to size of initial population; *B*, according to stage of maturity
of plant at time of infestation.

by those having smaller initial populations, and that production of
alate forms began earlier with a decrease in plant size at colonization.
The time of peak production of alate forms followed similar trends.

DISCUSSION AND INTERPRETATION OF RESULTS

The exact nature of the factors responsible for the results obtained
in this study is not entirely clear. Other investigators have found
several environmental factors that influence the production of winged

aphids, such, for instance as light, temperature, crowding, starvation, wilting of the host plant, parentage, and the use of certain chemicals in watering the host plant.   Ewing (*2*) has reviewed these factors as reported up to 1926.   Wadley (*9*), in work with *Rhopalosiphum prunifoliae* (Fitch), found that nutrition, as well as parentage and temperature, was an important factor.   Davidson (*1*) reported that winged migrants of *Aphis rumicis* L. developed to some extent as a result of an inherent established tendency, but that the actual number that occurred in any given generation was influenced by overcrowding and the condition of the food plant.   He also found that parentage was a significant factor because alate dispersal forms are usually produced by apterous females, and their occurrence in any given generation is affected by overcrowding, nutrition, and temperature.   Under suitable conditions of temperature and light, alienicolae generations may be maintained for long periods.

From studies with the mealy plum aphid (*Hyalopterus pruni* (Geoff.)), Smith (*8*) found that the number of drops of excrement produced by individuals per hour decreased as temperature increased. This would indicate that fluid intake was greater at lower temperatures, or that evaporation from the body of the insect was greater at higher temperatures. Smith found a high positive correlation between mean temperature 4 days before birth and the percentage of alate forms produced.   He also found that the number of nymphs deposited per day by individuals decreased as temperature increased, and suggested that the fundamental effect of temperature in these findings might be translated into terms of starvation.

Schaefer (*3*) found that a higher percentage of the progeny of the pea aphid (*Macrosiphum pisi* (Kltb.)) became alate when apterous adults were confined to wilted plants than when nymphs in the fourth instar were similarly treated.   Like results were obtained when adults and fourth-instar nymphs were starved for 12 or 24 hours and then returned to succulent plants for 46 hours.   Schaefer's studies indicated that the mechanism governing wing production is influenced by the concentration of waste products in the body of the mother aphid during the period of wing determination.   These waste products were in the form of proteins and carbohydrates, and their accumulation was thought to initiate wing development.   The concentration of body contents, hence of waste products, increased when the body fluid was insufficient to eliminate the waste products.   .

Shull (*6*) found that rudiments of wings were present during the embryonic development of all specimens of the potato aphid (*Macrosiphum solanifolii* (Ashm.)), regardless of whether embryos were destined to become alate or apterous adults.   These rudiments could be seen in first-instar nymphs, even in those destined to become wingless adults.   His earlier studies (*5*) showed that wing determination occurs within the last 16 to 34 hours before the young aphid is deposited, and his later investigations (*6*) showed that wing differentiation begins about 10 to 12 hours after wing determination.   The latter period varied with the changes in light and temperature to which the mother aphid was subjected.   Shull's subsequent studies (*7*) indicated that light and heat control wing production through the same mechanism, and he suggested that if a wing-suppressive substance is present it is produced when the mother aphid is subjected to high temperatures, continuous light, or a combination of the two factors.

In the present study several factors may have operated to influence the results (table 1 and figure 4). The more important of these appear to be plant size, density of aphid population, and plant condition. Although the populations of apterous individuals were not determined after colonization, no outstanding differences between subclasses were observed in ratios of alate to total aphid population per plant. Other factors, such as temperature and light, probably had no direct effect, but they may have had an indirect effect by accentuating differences in the physiological condition of plants and insects arising from the action of the principal factors.

Undoubtedly there were large differences in the food supply per aphid under the conditions that prevailed during the experiment. Marked differences, both in the size of plants of each group (figs. 1 and 3) and in the number of aphids placed on the plants, resulted in large differences in area of feeding surface and in the quantity of food available to each aphid. In some instances, at least, these differences were accentuated as the study progressed. Operating singly or together, these factors may have affected the quantities of plant fluid ingested and waste products excreted by the aphids and thus have influenced the number of alate aphids that developed.

Differences in plant condition could have resulted also from the fact that equal amounts of water were supplied to all plants regardless of their size or the density of the aphid population. On the hotter days some of the larger plants became semiwilted, and this may have affected existing nutritional differences arising from other factors.

SUMMARY

An experiment was conducted in the greenhouse to determine the importance of wild rutabaga (*Brassica campestris* L.) as a host of the green peach aphid (*Myzus persicae* (Sulz.)). The study was concerned primarily with the production of winged, or alate, forms when two variables were involved, namely, the stage of maturity of the plant at the time of infestation, and the size of the initial aphid population. Plants in four stages of growth were employed—very young, young, early-flower, and mature; and the initial infestation consisted of 1, 10, and 100 apterous adults on equal numbers of plants of each stage. From about 2 weeks after colonization, at frequent intervals until death, each plant was examined for winged aphids.

The fewest alate forms developed on the very young plants, principally because these plants were too small to support the initial population. When the original infestation consisted of 1 aphid per plant there was no real difference in the number of alate forms developed on plants of the other 3 stages of growth; but when it consisted of 10 aphids per plant, the number developing on young plants was larger than on either the early-flower or the mature plants; and when the infestation consisted of 100 aphids the number of alate forms developing on early-flower plants was almost significantly greater than on young plants.

Among plants of the same stage of maturity, the number of alate forms recovered from young plants was larger when the initial population was 10 aphids per plant than when it was 1 or 100. Larger numbers of alate forms were recovered from early-flower plants originally populated with 100 per plant than from those populated with 1.

The differences in average numbers recovered from mature plants of each subclass were not significant. In general, the time when production began, the rate of production, and the time of peak production of alate forms appeared to be associated negatively with an increasing order in plant size and positively with an increasing order in size of initial aphid population.

It appears probable that the principal factors influencing the production of winged forms in this experiment were size of plants, intensity of aphid infestation, and condition of the plants resulting from the uniform watering procedure employed. Irrespective of the probable factors involved, however, it is evident that wild rutabaga is an important host of the green peach aphid and that it may constitute a serious source of infestation if permitted to grow in or near potato fields.

LITERATURE CITED

(1) DAVIDSON, J.
    1929. ON THE OCCURRENCE OF THE PARTHENOGENETIC AND SEXUAL FORMS IN APHIS RUMICIS L., WITH SPECIAL REFERENCE TO THE INFLUENCE OF ENVIRONMENTAL FACTORS. Ann. Appl. Biol. 16: 104–134, illus.
(2) EWING, H. E.
    1926. WING PRODUCTION IN PLANT LICE. Amer. Nat. 60: 576–579.
(3) SCHAEFER, C. W.
    1938. PHYSIOLOGICAL CONDITIONS WHICH PRODUCE WING DEVELOPMENT IN THE PEA APHID. Jour. Agr. Res. 57: 825–841, illus.
(4) SHANDS, W. A., BRONSON, T. E., and SIMPSON, G. W.
    1942. BRASSICA CAMPESTRIS L. AND RAPHANUS RAPHANISTRUM L. AS BREEDING HOSTS OF THE GREEN PEACH APHID. Jour. Econ. Ent. 35: 791–792.
(5) SHULL, A. F.
    1928. DURATION OF LIGHT AND THE WINGS OF THE APHID MACROSIPHUM SOLANIFOLII. Arch. f. Entwickl. Mech. der Organ. 113: 210–239.
(6) ———
    1938. TIME OF DETERMINATION AND TIME OF DIFFERENTATION OF APHID WINGS. Amer. Nat. 72: 170–179, illus.
(7) ———
    1942. THE MECHANISM THROUGH WHICH LIGHT AND HEAT INFLUENCE GENETIC FACTORS FOR WING DEVELOPMENT IN APHIDS. Jour. Expt. Zool. 89: 183–195.
(8) SMITH, L. M.
    1937. GROWTH, REPRODUCTION, FEEDING, AND WING DEVELOPMENT OF THE MEALY PLUM APHID IN RELATION TO CLIMATIC FACTORS. Jour. Agr. Res. 54: 345–364, illus.
(9) WADLEY, F. M.
    1923. FACTORS AFFECTING THE PROPORTION OF ALATE AND APTEROUS FORMS OF APHIDS. Ent. Soc. Amer. Ann. 16: 279–303, illus.

THE ASSOCIATION OF HYLURGOPINUS RUFIPES WITH THE DUTCH ELM DISEASE PATHOGEN [1]

By K. G. PARKER, *associate professor*, LYLE E. HAGMANN, formerly *assistant*, DONALD L. COLLINS, formerly *assistant professor*, L. J. TYLER, *professor*, and HENRY DIETRICH, *assistant professor, New York (Cornell) Agricultural Experiment Station*, and W. E. OZARD, *horticultural inspector, Bureau of Plant Industry, New York State Department of Agriculture and Markets* [2]

INTRODUCTION

There seems to be general agreement that in the United States the bark beetle *Scolytus multistriatus* (Marsham) is the principal and most effective carrier of the Dutch elm disease pathogen, *Ceratostomella ulmi* (Schwarz) Buis. The disease occurs sporadically, however, in areas of New York State where this species is not known. The identification of the disseminating and inoculating agent or agents in such areas is the objective of the studies described herein.

Most of the field observations reported in this paper were made northeast of the City of Binghamton, N. Y., in Broome and Chenango Counties, where the European elm bark beetle *Scolytus multistriatus* has not been found, but where the native elm bark beetle *Hylurgopinus rufipes* (Eich.) is prevalent. Laboratory studies correlated with the field work were made chiefly with material collected in this area.

REVIEW OF LITERATURE

Britton (4)[3] and Clinton and McCormick (7) called attention to the fact that *Hylurgopinus rufipes* might transmit the Dutch elm disease to healthy trees. They isolated *Ceratostomella ulmi* from beetles of this species taken from the bark of a diseased tree in the field, and they found that when beetles from diseased trees were confined in test tubes with twigs cut from healthy elm trees the beetles chewed the bark and the fungus fruited on the twigs. These workers observed that *Scolytus multistriatus* was not present at Old Lyme, Conn., but that *H. rufipes* was abundant on a diseased tree found there in 1934. Britton stated that *H. rufipes* may make feeding injuries on elm twigs. Kaston and Riggs (*13*) expanded these observations and suggested that the subsequent spread of the disease at Old Lyme probably was due to feeding by this species. They reported that it made feeding tunnels in the bark on branches as small as 1 inch in diameter, or

even smaller, but they did not observe injury to the wood. Becker (*2, 3*) previously had reported bark tunnels on branches of that size similar to the winter tunnels in which he had occasionally observed injury to the wood.

Collins (*8*) reported an experiment in which 1 out of 11 elm trees became infected the year after adults of *Hylurgopinus rufipes* were confined on the trunks in the fall. Hagmann [4] confined fungus-bearing beetles on trees 1 to 4 inches in diameter during May and June and found infection from feeding injuries on the trunk near the origin of the first branch, or on the branches themselves, in 3 of 19 cages.[5] Collins stated that the hibernating adults, by boring into the tree in the spring, make contact with the wood, but that this is done before new wood vessels are produced and infection would not be expected at that time. He believes that adults arising later in the spring from overwintering larvae would make similar injuries to the wood after new vessels are present and that infection would then be expected. This apparent relation of time of inoculation to probability of infection seems to be supported by inoculation experiments (*6, 15*).

Several workers have observed that the adults of *Hylurgopinus rufipes* may feed on small branches as well as large soon after they emerge, but there is a difference of opinion regarding the relative numbers of the beetles that overwinter as larvae and as hibernating adults (*12, 14*), and therefore there is some question as to just when most of the feeding occurs.

Collins et al. (*9*) found that after emerging from diseased trees *Hylurgopinus rufipes* may carry the fungus pathogen into uninfected dead or dying elm material when making maternal galleries. Jones and Moses (*11*) isolated the fungus from substantial numbers of adults collected as they were attracted to felled healthy elm trees at different locations in New Jersey and in the Hudson River Valley in New York. The writers made studies, not reported in detail, similar to those of Collins et al. and to those of Jones and Moses, with similar results.

CULTURE STUDIES

EXPERIMENTAL PROCEDURE

Elm logs infested with the beetles were cut from diseased trees, or uninfested branches were cut from diseased trees and exposed to beetle attack in the field, and then placed in cages out of doors. The emerging adults were collected and isolation trials made for *Ceratostomella ulmi*. Cultures were made aseptically by crushing the beetles in water in petri dishes and mixing the macerated tissue with melted potato-dextrose agar.

In a similar manner, naturally infested material apparently not killed by the Dutch elm disease (such as cut wood, broken branches, and branches or trees dying because of poor environmental condi-

---

[4] HAGMANN, LYLE E. FEEDING HABITS AND RELATED ACTIVITIES OF THE TWO ELM SCOLYTIDS, SCOLYTUS MULTISTRIATUS (MARSHAM) AND HYLURGOPINUS RUFIPES (EICHHOFF) WITH REFERENCE TO THE SPREAD OF THE DUTCH ELM DISEASE PATHOGEN CERATOSTOMELLA ULMI (SCHWARZ) BUISMAN. 1945. [Unpublished doctor's thesis. Copy on file Cornell University library, Ithaca, N. Y.]

[5] In other work (unpublished) done at the Cornell University Dutch elm disease laboratory at Yonkers, N. Y., infection was obtained in a similar way on small potted trees.

tions) was collected in the field, placed in cages, and the emerging adults cultured.

For a more rapid method of determining the presence or absence of the fungus on beetles in the field, callow adults and pupae were collected in the field and cultured aseptically. These were obtained from cut wood and naturally occurring dead wood which apparently had not been killed by the Dutch elm disease.

A study was also made to determine whether the fungus would persist on hibernating adults. Beetles were taken from cages containing logs from which adults bearing the fungus had been cultured, placed on cut healthy logs late in the fall, and stored over the winter in small screen cages out of doors. The following spring the overwintered live beetles were dug out of the logs and cultured. Hibernating adults collected in the field during the winter and spring were also cultured.

BEETLES EMERGING FROM WOOD CUT FROM DISEASED TREES

In 1937, wood not infested with beetles was cut from diseased trees and stored at 5° C. In the spring of 1938, it was exposed in the field to beetle entry for egg laying at Armonk, Westchester County, N. Y. and on July 8 was placed in emergence cages out of doors at Yonkers.

On July 13, 1940, material infested with *Hylurgopinus rufipes* was cut from a diseased tree at Sanford, Broome County, N. Y., and on August 3 it was placed in an emergence cage out of doors. The results of cultures made from *H. rufipes* adults that emerged from the two sets of material are shown in table 1.

TABLE 1.—*Results of cultures made from Hylurgopinus rufipes adults emerging from wood from diseased trees infested in the field*

Source of wood	Period of collection of beetles for culturing	Beetles		
		Total	With *C. ulmi*	
			Number	Percent
Diseased wood infested at Armonk, N. Y. _____	July 26–Aug. 7, 1938___	261	32	12.3
Infested wood from diseased tree at Sanford, N. Y__	Aug. 8–Aug. 16, 1940__	38	30	78.9

BEETLES EMERGING FROM NATURALLY INFESTED MATERIAL FROM NONDISEASED TREES

In 1940, in connection with a survey to determine the incidence of *Ceratostomella ulmi* on *Scolytus multistariatus* emerging from naturally infested material in Westchester County during the late summer, *Hylurgopinus rufipes* also was obtained from many of the samples. Eight to 451 (usually about 150) adults of the latter species were cultured from each of 41 wood collections, which consisted of 3 logs each 4 to 5 inches in diameter and about 16 inches long. The fungus was obtained from beetles from 7 of the 41 stations.

During the same summer similar survey material was collected in the disease area in Broome and Chenango Counties. The fungus was obtained from *Hylurgopinus rufipes* adults emerging from 4 of the 11 collections.

ADULTS AND PUPAE COLLECTED IN THE FIELD

In 1941, collections from infested material were made at various stations in Broome and Chenango Counties. Adults or pupae, or both, were taken as they were available at the time the station was visited. Table 2 gives the results of cultures made from beetles collected within or at the edge of the area of disease incidence as known in 1941. Beetles carrying the fungus were obtained at 7 of the 14 stations. At these 7 stations 98 adults and pupae of a total of 1,297 gave positive cultures.

TABLE 2.—*Results of cultures made from Hylurgopinus rufipes collected from infested material in the field in 1941*

Site No.	Number of bark samples	Total area of bark sampled (square feet)	Beetles cultures (adults and pupae)			Number bark samples from which beetles yielding positive cultures emerged
			Total	With *C. ulmi*		
				Number	Percent	
1	4	3 6	200	41	20 5	4
2	4	10 2	203	14	6 9	4
3	4	4.0	200	6	3 0	2
4	4	2 7	199	0	0	0
5	4	.7	198	2	1 0	1
6	1	6.0	50	0	0	0
7	1	6 0	96	14	14. 6	1
8	4	18. 0	201	0	0	0
9	4	15 9	200	2	1 0	1
10	4	7 9	200	19	9 5	2
11	4	4 5	212	0	0	0
12	1	5 0	99	0	0	0
13	4	7. 5	201	6	0	0
14	1	10 0	24	0	0	0

PERSISTANCE OF FUNGUS ON HIBERNATING ADULTS

From September 30 to November 10, 1936, beetles emerging from material from diseased trees (cages Rh 29 and Rh 30) were introduced into cages out of doors (cages Rh 36 and Rh 37) containing wood freshly cut from healthy elm trees in which these beetles could hibernate. On April 15, 1937, beetles that had lived through the winter were collected from this wood for culturing. At that time they were just beginning to make maternal galleries. The dead beetles were not cultured. The data gathered in this experiment are presented in table 3.

TABLE 3.—*Comparison of results of cultures of adults of Hylurgopinus rufipes emerging from diseased wood in the fall and from hibernation in the spring*

Source of beetles (emergence cage No.)	Results of culturing beetles from original source material, fall of 1936			Source of beetles (hibernation cage No.)	Number beetles introduced, fall 1936	Results of culturing beetles from hibernation cages, spring of 1937		
	Number cultured	Number with *C. ulmi*	Percent with *C. ulmi*			Number cultured	Number with *C. ulmi*	Percent with *C. ulmi*
Rh 29	129	73	56. 6	Rh 36	193	19	11	57. 9
Rh 30	169	62	36.7	Rh 37	130	11	7	63 6

At intervals during the winter of 1940–41 and the spring of 1941 beetles were collected from their hibernation galleries in the field and cultured. All collections were made within the known area of disease incidence in Broome and Chenango Counties from the trunks and large branches of healthy trees. The time of year each collection was made may be roughly determined from the date on which the cultures were made. This was within 30 days of the time of collection of the beetles. Information on these experiments is given in table 4.

TABLE 4.—*Cultures of hibernating adults of Hylurgopinus rufipes collected from healthy trees in the field*

Month of culturing	Number of stations	Number of beetles	Number of cultures	Number of cultures with *C ulcei*
November 1940	1	43	43	0
December 1940	7	188	188	4
February 1941	9	110	110	1
May 1941	8	146	36	2
June 1941	8	213	67	0

FIELD OBSERVATIONS IN THE UPSTATE AREA OF DISEASE INCIDENCE

Following the discovery in 1939 of the Dutch elm disease in Broome County, N. Y., by scouts of the United States Department of Agriculture, detailed observations were made on many of the infected trees found in that area. These studies were similar to those made in the Hudson River Valley (10). Since *Scolytus multistriatus* is not known to occur in the Broome County area, and it has been shown that *Hylurgopinus rufipes* in that area carries the fungus and can inoculate healthy trees, it is probable that the latter species inoculated all the diseased trees that were observed.

In 1940, 56 diseased trees were observed at 30 sites. In 34 of these trees it was estimated that at least 10 percent of the branches were dead and had harbored or were still harboring *Hylurgopinus rufipes*. In addition, unsuccessful maternal galleries had been attempted in at least 4 other trees.

In 1941, 30 diseased trees were observed at 22 sites. In 15 of the trees, at least 10 percent of the wood was dead.

At each site an attempt was made to determine the year when each tree first became infected and the nearest source of beetles. This particular observation was independent of the subsequent history of the diseased trees. That is, the beetle emergence referred to here occurred prior to infection of the trees.

Table 5 lists the types of wood which contained the observed beetle sources and the distances from the diseased trees to the nearest probable beetle sources. In some instances no source was found. The observed emergence usually, but not necessarily always, occurred at a time when the beetles could have made the specific inoculation involved. In most of the diseased trees cited as being their own sources of beetles, · the branches from which beetles had emerged

were killed by unfavorable soil conditions or other environmental factors. Such trees are included among those listed as being within 100 feet of the source of beetles.

TABLE 5.—*Types of beetle sources and number of diseased trees associated with them, and distances from beetle sources to diseased trees*

Type of beetle source and distance from source to diseased trees	Number of diseased trees associated with beetle sources		
	1940	1941	Total
Types of beetle source:			
Branch of same diseased tree [1]	24	5	29
Other diseased trees [2]	8	2	10
Dead trees or dead branches in other trees	21	10	31
Broken-off branches or logs	0	5	5
Source unknown	3	8	11
Distances from beetle source to diseased trees:			
100 feet or less	36	15	51
Between 100 and 250 feet	12	7	19
Between 250 and 500 feet	5	0	5
Unknown	3	8	11
Total trees observed	56	30	86

[1] Beetles emerged before tree became infected.
[2] Only sources of beetles observed that are believed to have been diseased branches. All others listed in this table were in material not killed by the Dutch elm disease.

Feeding tunnels as described by Kaston (*12*) and others were observed on many of these trees. Many tunnels reached and injured the wood, especially on the smaller branches, ¾ inch to 4 inches in diameter, where the bark was thin. Injury to the wood was also observed apparently arising from hibernation galleries as described by Becker (*2*). The appearance of the injury and adjacent recent extension to the hibernation tunnel when seen in the spring usually indicated that wood injury was made at that time.

A rather high proportion of the diseased trees contained live colonies of *Hylurgopinus rufipes*. It is suggested that many such trees were inoculated by the beetles in connection with oviposition in portions which were still in a state of suitable physiological activity to permit infection at the time of the actual or attempted oviposition. There are no published data demonstrating that infection results from tunnels made for egg laying, but it has been shown that trial entries by *Scolytus multistriatus* may result in effective inoculations.[6] On trees with completed galleries of *H. rufipes*, trial entries or feeding tunnels which injured the wood were sometimes found in adjacent, somewhat more vigorous branches.

The incidence of diseased trees in this area was very low, an average of one in 5 square miles in 1940 and one in 10 square miles in 1941. Frequently, the disease attacked the weakest tree in a group. That is, trees receptive to beetle entrance became diseased first. Later, perhaps the following year as a rule, more vigorous trees in the neighborhood became diseased. Probably, in diseased tree associations of this kind the first inoculations were made by trial entries for egg laying,

[6] Unpublished work at Cornell University Dutch elm disease laboratory at Yonkers, N. Y.

as in observations reported by Collins (*8*). Subsequently, the bettle population was built up to such an extent that the relatively small percentage making injuries to the wood in feeding activities was sufficient to make a few effective inoculations. .

DISCUSSION

The following discussion is an attempt to interpret the results of these studies, and the low incidence of the Dutch elm disease in the area northeast of Windsor, N. Y., where the bark beetle species *Scolytus multistriatus* is absent but *Hylurgopinus rufipes* is present, in the light of published information on the life history and habits of the latter species. Since *Ceratostomella ulmi* was obtained from *H. rufipes* adults taken from cut or broken elm branches originating in nondiseased trees, the breeding activities of this species is probably a means of introducing the fungus into new areas. The abundance of the fungus in such material in the field was substantially as great as that found by Collins et al. (*10*) associated with *S. multistriatus;* that is, it was obtained from beetles from similar percentages of wood samples, and the number of fungus colonies per beetle was about the same.

Probably the time when inoculations are most likely to cause destructive infections is early in the spring, but not until after new xylem vessels are produced. Any one of three methods of inoculation by *Hylurgopinus rufipes* might be operative at this time. Many of the wood injuries resulting from extensions of hibernating tunnels and from attempted entries by overwintered adults may be made before new xylem vessels are produced and such injuries would not be likely to result in infection. However, according to Kaston (*12*), some of these beetles do not leave their hibernation tunnels until late May or June, and infection should result from inoculations made by them. Feeding by beetles that arise from overwintering larvae has been shown to cause infections. There is also the possibility that effective inoculations may be made in the middle of the summer or later by this species. The work of Banfield (*1*) indicates that the movement of the fungus spores when introduced into trunks or large branches during the latter part of the growing season is slower and less extensive than earlier in the season. But the movement is extensive enough to make it appear plausible that inoculations made either by trial entry or by feeding by *Hylurgopinus rufipes* late in the season might be effective. Furthermore, since this species feeds in larger branches, inoculations made in the summer would be more likely to result in infection than would those made at the same time by the feeding of *Scolytus multistriatus* in the small twigs. One of the writers (Tyler) [7] found that hypodermic inoculations on the trunks late in the season were more successful than those made on the twigs at the same time.

Regardless of the several possible methods by which *Hylurgopinus rufipes* may make inoculations, the number of known infections in areas in New York State where it is abundant and *Scolytus multistriatus* is absent are relatively few (*5*). This fact is undoubtedly associated with two well-defined relationships, namely, that (1) egg-laying trial

---

[7] Unpublished data.

tunnels apparently are usually made in branches with insufficient vigor for the fungus to invade the live parts of the trees, and (2) wood injuries made in connection with hibernation and feeding activities are apparently not sufficiently frequent, or are made at the wrong time of the year, to account for many inoculations. Detailed observations by the writers on nearly 100 diseased trees and more casual observations over a period of several years on many more have failed to reveal abundant injuries of any of these types on sound branches. When compared with the deep-feeding injuries to the twigs made by *S. multistriatus* they would be considered as rare.

SUMMARY

The bark beetle *Hylurgopinus rufipes* may carry the Dutch elm disease pathogen when emerging from infested wood originating in diseased trees, cut elm wood, or dead branches and trees. The fungus may survive the winter on hibernating adults. It seems to be adapted to a congenial association with this native beetle in dead elm wood.

Field observations on the incidence of diseased trees in New York State indicate that *Hylurgopinus rufipes* has been very much less effective as an inoculating agent in the area studied than *Scolytus multistriatus* in the Hudson River Valley. One reason for this is the fact that *H. rufipes* is much less likely than *S. multistriatus* to make injuries that reach to and into the wood of sound trees and branches.

LITERATURE CITED

(1) BANFIELD, W. M.
    1941. DISTRIBUTION BY THE SAP STREAM OF SPORES OF THREE FUNGI THAT INDUCE VASCULAR WILT DISEASE OF ELM. Jour. Agr. Res. 62: 637–681, illus.
(2) BECKER, W. B.
    1935. SOME OBSERVATIONS ON THE OVERWINTERING HABITS OF THE AMERICAN ELM BARK BEETLE, HYLURGOPINUS RUFIPES EICH. Jour. Econ. Ent. 28: 1061–1065, illus.
(3) ――――
    1937. SOME NOTES ON THE TUNNELING HABITS OF HYLURGOPINUS RUFIPES EICH. Jour. Econ. Ent. 30: 375.
(4) BRITTON, W. E.
    1935. ANOTHER PROBABLE CARRIER OF THE DUTCH ELM DISEASE. *In* Connecticut State Entomologist Thirty-fourth Report 1934. Conn. (State) Agr. Expt. Sta. Bul. 368: 256–257. illus.
(5) BUCHHOLZ, A. B.
    1943. CONTROL OF DUTCH ELM DISEASE. *In* Report of Bureau of Plant Industry. N. Y. State Dept. Agr. and Markets Ann. Rpt. 1942: 137–143, illus.
(6) BUISMAN, C.
    1934. VERSLAG VAN DE ONDERZOEKINGEN OVER DE IEPENZIEKTE, VERRICHT IN HET PHYTOPATHOLOGISCH LABORATORIUM WILLIE COMMELIN SCHOLTEN TE BAARN GEDURENDE 1933. Tijdschr. over Plantenziekten 40: [65]–87.
(7) CLINTON, G. P., and McCORMICK, F. A.
    1935. THE DUTCH ELM DISEASE, GRAPHIUM ULMI, IN CONNECTICUT. Science 81: 68–70.
(8) COLLINS, C. W.
    1941. STUDIES OF ELM INSECTS ASSOCIATED WITH DUTCH ELM DISEASE FUNGUS. Jour. Econ. Ent. 34: 369–372.

(9) ————, BUCHANAN, W. D., WHITTEN, R. R., and HOFFMANN, C. H.
      1936. BARK BEETLES AND OTHER POSSIBLE INSECT VECTORS OF THE DUTCH
            ELM DISEASE CERATOSTOMELLA ULMI (SCHWARZ) BUISMAN. Jour.
            Econ. Ent. 29: 169–176.
(10) COLLINS, D. L., PARKER, K. G., and DIETRICH, H.
      1940. UNINFECTED ELM WOOD AS A SOURCE OF THE BARK BEETLE (SCOLYTUS
            MULTISTRIATUS MARSHAM) CARRYING THE DUTCH ELM DISEASE
            PATHOGEN.   N. Y. (Cornell) Agr. Expt. Sta. Bul. 740, 14 pp.
(11) JONES, T. H., and MOSES, C. S.
      1943. ISOLATION OF CERATOSTOMELLA ULMI FROM INSECTS ATTRACTED TO
            FELLED ELM TREES.   Jour. Agr. Res. 66: 77–85, illus.
(12) KASTON, B. J.
      1939. THE NATIVE ELM BARK BEETLE HYLURGOPINUS RUFIPES (EICHHOFF)
            IN CONNECTICUT.   Conn. (State) Agr. Expt. Sta. Bul. 420, 39
            pp., illus.
(13) ———— and RIGGS, D. S.
      1938. ON CERTAIN HABITS OF ELM BARK BEETLES.   Jour. Econ. Ent. 31:
            467–469, illus.
(14) MARTIN, C. H.
      1938. FIELD NOTES ON THE LIFE HISTORY OF HYLURGOPINUS RUFIPES
            (EICH.).   Jour. Econ. Ent. 31: 470–477, illus.
(15) TYLER, L. J., PARKER, K. G., and POPE, S.
      1940. RELATION OF WOUNDS TO INFECTION OF AMERICAN ELM BY CERATO-
            STOMELLA ULMI, AND THE OCCURRENCE OF SPORES IN RAINWATER.
            Phytopathology 30: 29–41.

·

A STUDY OF MEIOSIS IN THE MICROSPOROCYTES OF INTERSPECIFIC HYBRIDS OF SOLANUM DEMISSUM × SOLANUM TUBEROSUM CARRIED THROUGH FOUR BACKCROSSES [1]

By LEONA O. SCHNELL [2]

Formerly *assistant in plant breeding, New York (Cornell) Agricultural Experiment Station*

INTRODUCTION

Many studies on the cytology of *Solanum* hybrids have been undertaken in an attempt to solve the problems of sterility, abnormal segregations, nature of polyploidy, and various other phenomena connected with the breeding of potatoes. Despite the many contributions, little is known of the basic causes of these observed abnormalities.

The present study was undertaken at the suggestion of Dr. Donald Reddick, of Cornell University, who has long been engaged in breeding for blight resistance in the potato by crossing the wild Mexican species *S. demissum* L. (somatic chromosome number=72) with *S. tuberosum* L. (somatic chromosome number=48) in an attempt to retain the commercial characters of *S. tuberosum* and add the blight resistance of *S. demissum* to the stock. This has proved difficult in many ways. With the failure of the expected segregations in the F_2 generation, the backcross method was used, and rigid selection for plants combining the desired characteristics was effected. In general the results were similar to those obtained early in the breeding program by Dr. Reddick and Dr. C. H. Myers, of the Department of Plant Breeding, with species other than *tuberosum*. Their findings hitherto unpublished [3] are as follows:

> *S. demissum* × *S. maglia*
> F_1: Plants all immune
> F_2: 15 plants. all immune
> BC by *maglia*: 19 plants; 10 immune, 9 susceptible

Journal of Agricultural Research, Washington, D. C.

Vol. 76, Nos. 9, 10
May 1, 15, 1948
Key No. N. Y. (Cornell)—60.

(185)

sidered impracticable to continue the work. In this paper, then, only the behavior of the chromosomes in the meiotic divisions of hybrids through four backcrosses will be discussed.

DESCRIPTION OF RESULTS

The description of results will give mainly the cytological phenomena observed in *S. demissum, S. tuberosum*, and their hybrid progeny, together with observations on their characteristic breeding behavior. It will therefore be presented largely as an explanation of the illustrations, more extended comment being reserved for a later section of the paper.

SOLANUM DEMISSUM

Figure 1, *A*, represents an early diakinesis stage in which the nuclear membrane is still present and the chromosomes are not completely contracted. Two chromosomes are in contact with the nucleolus. A few univalents may be distinguished; bivalents and trivalents are in the majority, but quadrivalents and one hexavalent may also be seen. These configurations are characteristic of the chromosomes of *Solanum* at this particular stage of contraction (*2, 6, 8, 11, 12*). With further contraction and added nucleic acid, the connecting strands which are visible at this stage assume shapes similar to those in figure 5, *F*, which represents a late diakinesis or prometaphase stage.

Figure 1, *B*, shows the alignment of chromosomes on the metaphase plate, with trivalents, bivalents, and an occasional univalent clearly detectable.

Figure 1, *C*, shows an anaphase stage with regularity of chromosome movement indicated.

Figure 1, *D*, represents an interkinesis; the number of chromosomes is unequal in the two nuclei; but, again, bivalents and trivalents comprise the greater number of associations, with one quinquevalent evident. Presumably these associations are not retained in regular divisions. There is some question as to their origin; they may be the result of nonrandom segregation or of a reuniting of ends of chromosomes. Since on the anaphase spindles in *demissum* no evidence of retention of connecting chromatids is indicated, it would seem that a reuniting had occurred. Two nucleoli are visible in the nucleus to the right.

Figure 1, *E*, shows a second anaphase which is proceeding regularly.

Figure 1, *F*, shows the 4 immature spores delimited by walls. No groups of more than 4 spores were found at this stage on any of the slides. Each of the pollen grains would be expected to contain 36 chromosomes if all divisions were regular and segregation of chromosomes were random. The exact number is not represented in the figure shown. In the upper left-hand nucleus, associations of 5 and 7 chromosomes are seen. The remaining 3 nuclei show associations in lesser numbers.

SOLANUM TUBEROSUM

Figure 2, *A–P*, represents three varieties of *S. tuberosum*, one pollen-sterile and two pollen-fertile types.
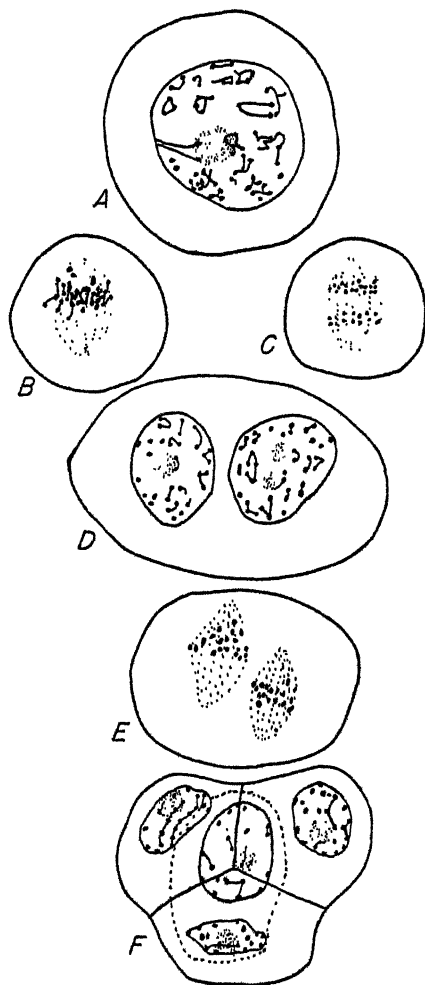
FIGURE 1.—*Solanum demissum:* *A*, Early diakinesis; *B*, metaphase; *C*, first anaphase; *D*, interkinesis; *E*, second anaphase; and *F*, immature tetrad.

KATAHDIN

Figure 2, *A–D*, inclusive, shows some of the irregularities found in a pollen-fertile variety, the divisions of which for the most part proceed quite regularly with the delimitation of four spores.

Figure 2, *A*, shows an early diakinesis stage with univalents, bivalents, trivalents, and a quadrivalent. Both ring and chain configurations among the tri- and quadrivalent forms are seen.

Figure 2, *B*, is a metaphase in which the chromosomes have for the most part lined up on the plate, but a few univalents and one bivalent have failed to do so.

Figure 2, *C*, is a late anaphase with chromosome bridges in evidence. The chromosomes at either pole have definite connection such as

those seen at diakinesis, as though there had been a segregation of chromosome groups rather than a randon segregation of single chromosomes. Disjunction of some members may occur, but nondisjunction in some cases is evident.

Figure 2, *D*, shows five nuclei in this sporocyte, but for the most part four nuclei are delimited in Katahdin.
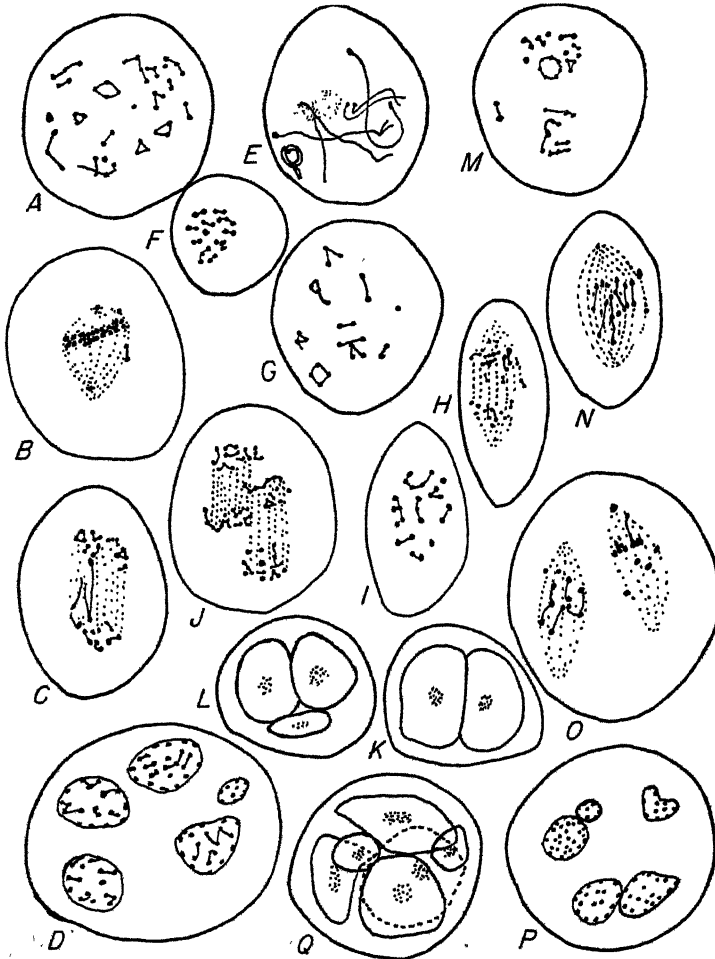
FIGURE 2.—*Solanum tuberosum.* *A–D*, Abnormalities in Katahdin: *A*, Diakinesis; *B*, first metaphase with lagging chromosomes; *C*, late anaphase with bridges and associations; *D*, sporocyte with five nuclei. *E–L*, meiosis in Russet Rural: *E*, Zygotene stage showing two nucleoli and an inversion; *F*, polar view of metaphase; *G*, early diakinesis with associations; *H*, irregular first anaphase; *I*, polar view at anaphase; *J*, irregular second anaphase; *K*, dyad; *L*, triad. *M–Q*, abnormalities in Earlaine: *M*, late first anaphase with associations; *N*, irregular first anaphase; *O*, abnormal second anaphase; *P*, sporocyte with five nuclei; *Q*, six spores including two microspores.

If environmental conditions are favorable for seed setting, the variety Katahdin is self-fertile and the pollen is effective in fertilizing other female-fertile varieties.

The variety Russet Rural, unlike Katahdin, is almost completely pollen-sterile, but it is receptive as a female to pollen from *S. demissum*. In this respect it is one of the few varieties of *S. tuberosum* favorable for use as a female parent when *S. demissum* pollen is used.

Figure 2, *E*, is a zygotene stage showing only a few of the chromosomes. It was chosen for illustration because of the configuration at the lower left, which might possibly have arisen as the result of an inversion in one of the chromosomes. Two nucleoli have been formed; this is frequent in Russet Rural, but not in Katahdin and Earlaine.

Figure 2, *F*, is a polar view of a metaphase which has 24 chromosomes, the expected number following the first division. Associations may be seen to involve nearly all of them.

Figure 2, *G*, shows some of the associations of early diakinesis, including uni-, bi,- tri-, and quadrivalents in rings and chains. The configuration which would appear to be a quinquevalent is in reality a bi- and a trivalent lying at different levels in the nucleus.

Figure 2, *H*, is a first anaphase with many irregularities, including lagging bivalents or dividing univalents; it is difficult to say which is the case. Dividing univalents are often seen on the first anaphase spindle in *Solanum* hybrid meiotic divisions (*6, 8*). It would appear that some of the bivalents are being drawn intact to either pole.

Figure 2, *I*, is another polar view at anaphase chosen because of the associations present, the chain of 5 and the evident association of a trivalent and a bivalent being totally unexpected in a tetraploid individual if one considers these associations to be secondary and due to homology of chromosomes. Again, the number of chromosomes is 24, which would be expected normally in Russet Rural following the first division.

The second divisions very frequently fail entirely in this variety, and, when present, are of the type seen in figure 2, *J*, with very evident associations of varying numbers of chromosomes.

Regardless, however, of the success or failure of the second division, the pollen that is formed appears to be entirely devoid of functional cytoplasm, although shrunken protoplasm can usually be distinguished in some portion of the pollen grain. Dyads, triads, and higher, groupings are present, but all are functionless. Figure 2, *K* and *L*, shows a dyad and a triad, respectively.

Figure 2, *M*, *Q*, inclusive, are from the variety Earlaine.

Figure 2, *M*, is a late first anaphase approaching interkinesis in which a ring of seven chromosomes is visible. The bivalent to the left will probably be excluded from the nuclei.

Figure 2, *N*, is an early anaphase with evidence of difficulty in breakage of the connecting chromatids; one member of the group remained at the pole or was precocious in movement.

Figure 2, *O*, is a second anaphase showing about the same type of abnormality. These chromosomes were on the upper side of the spindle, but, presumably, were the other side in evidence, the same types of irregularity would be observed.

Figure 2, *P* and *Q*, are of nucleus formation and pollen groups, respectively. One micropollen grain is present in *P* and two micrograins in *Q*; yet, for the most part, Earlaine has good functional pollen.

FIRST GENERATION HYBRIDS

BREEDING BEHAVIOR

Speaking of the first-generation hybrids, Reddick (*15, p. 556*) states:

At Ithaca, thus far, *S. demissum* as pollen parent has given a very scant set of seeds only 5 times and each time on variety Rural or on Rural hybrids. The first-generation hybrids are intermediates in many characters, are all blight-immune, and are practically male-sterile. Less than 100 individuals of the second generation have been produced. A segregation occurs and blight immunity is found to be heritable, although it is not possible to determine the probable mode from such meager material. Also, some of the progeny have the general appearance of the Rural parent with fairly short stolons and tuber production before autumnal equinox.

When *Solanum demissum* is the female parent and fertile pollen from cultivated varieties is employed, the first-generation hybrids usually resemble the wild parent rather closely, although some intermediate characters may be observed. Practically all of the plants of this generation are immune from blight (90 per cent at present, but some of the *demissum*-like parents are of questionable genetic purity), they develop long to very long stolons, and a good many of them persist in the habit of producing tubers only with short day. Such hybrids are mostly fertile. In the second and succeeding generations (at least as far as the fourth) all plants tested have reverted to the wild type; all are immune from blight and continue so in succeeding generations.

The following excerpt is taken from a later paper of Reddick (*16, pp. 121–122*):

After a first cross has been effected there comes the problem of selfing some of the first generation hybrids. This is often very difficult or quite impossible to accomplish. Many such plants are self sterile. The easiest method is to plant a considerable number of seeds and search for plants which produce seeds in the field. Such seeds may be used safely since they are almost certainly self-fertilized; and in any event the result is almost always disappointing. The recombination and segregation of characters which ordinarily are expected rarely occurs. Instead, the second generation usually has the appearance and characters of *S. demissum*, the female parent of the first cross.

Because of the desirability of having some knowledge of the performance of the plants included in this study, the following notes from the field and greenhouse as taken by Reddick and Peterson are included:

FFA–1: [*S. demissum* (886)] × [860 (Early Ohio)]

Thirty-five immune plants were obtained, all of which were large and late except 1 or 2, which died early. The plants are dark green, drought-tolerant, and also frost-tolerant to 20° F. They bear large white tubers. In a breeding test, 2 pollinations set seed and 2 failed.

FEJ–1: [*S. demissum* (531)] × [245/25 (U. S. 444/12 × Jubel)]

Fifteen immune plants were secured from this cross, all of which were drought-tolerant. Some were frost-tolerant, but most of them frosted. None of the tubers were purple early, but by December all but 2 were purple. In 1944 in the greenhouse 2 pollinations set seed and 6 failed. In 1946, 1 pollination was successful.

FEK–1: [*S. demissum* (531)] × [245/86 (U. S. 444/12 × Jubel)]

This plant set seeds naturally in the field, but was unsuccessful as a male in crosses. It failed to set seed in 1944. In 1946, there were one set and six failures. The plants are drought-tolerant and excellent in appearance.

FEL–1: [*S. demissum* (531)] × [627/126 (Houma×Katahdin)]

Seventeen immune plants were obtained from this cross; 12 tubers produced large plants with a big crop of tubers ranging in size from eggs to marbles. The plants are drought-tolerant and late. In 1944 they failed 6 pollinations and set 1. In 1946 they failed 7 pollinations and set 5.

FEM–1: [*S. demissum* (531)] × [902/4 (*S. andigenum*)]

S. andigenum is a 48-chromosome South American species. The F_1 set seeds in the field. In 1944 3 pollinations failed. In 1946 there were 2 sets and 7 failures. The plants are pale green. Some tubers are tinged with purple. No scab was present. All plants were frost- and drought-tolerant.

FEQ–1: [*S. demissum* (531)] × [CDU/4 (Houma × Ostragis)]

Twelve immune plants were secured. In 1944 this plant set 1, failed 2 pollinations; in 1946 it set 1 and failed 4. Most of the plants are frost-tolerant, but there is variability. Most of the tubers have a purple tinge, but only white tubers were saved.

FEY–1: [*S. demissum* (886) × [245/25 (U. S. 444/12 × Jubel)]

Twenty-five immune plants were obtained. FEY–1 sets seeds naturally in the field. In 1944 it set 2 and failed 3 pollinations; in 1946 it set 5 and failed 5. The pollen from this plant will set on Rural. It is drought- and frost-tolerant with very long stolons and large tubers.

FFC–1: [*S. demissum* (886)] × [CDU/4 (Houma × Ostragis)]

Twelve immune plants were obtained which all looked alike in the field. All had white tubers and long stolons. The plants were large and dark green and drought- and frost-tolerant. In 1944 pollination with 5 types of pollen resulted in failure and 2 were successful. In 1946 the 1 pollination performed was a failure.

FFD–1: [*S. demissum* (886)] × [Jubel]

Five plants were obtained, four of which were immune and one susceptible. Three plants were large and green with white tubers and were frost- and drought-tolerant. They were barren in the greenhouse. In 1946, pollination with four types of pollen was effective and five were not.

CYTOLOGY

Figure 3, *A* (FEM–1), *B* (FEK–1), *C* (FEY–1), and J (FFA–1) are prophase stages. In *B* and *C* there are 2 nucleoli, a condition which is rather unusual in these hybrids. In *C* the mode of pairing on the right may indicate a translocation in 1 of the chromosomes. Figure 3, *B*, is a diakinesis stage, and *A* and *J* are an early diakinesis stage in which there is considerable variation in pairing. Figure 3, *J*, has many bivalents, whereas *A* has mostly higher degrees of valency. The plant from which *A* was derived is pollen-fertile, but the plant forming mostly bivalents is pollen-sterile. In no case have 12 univalents and 24 bivalents been seen, as was reported by Salaman (*17*) for his material. This would lead one to believe that pairing may take place not only between the *tuberosum* sets and the *demissum* sets, but also between chromosomes from the same parent, presumably the one which contributes the larger number of homologues. Cytological chromosome markers would be needed to decide this definitely.

Figure 3, *D* (FFA–1), *E* (FEJ–1), *G* (FFD–1), and *F* (FEJ–1) show successive stages in metaphase I and anaphase I. A polar view with 27 chromosomes is seen in figure *F*. If the 60 chromosomes present in the hybrid had been equally divided, 30 chromosomes would be expected in this polar view. Figure 3, *D*, shows several chromosomes out of the metaphase plate. The size of the chromosomes is interesting when compared with that in figure 3, *E*, for instance. The magnification is the same, yet there is an appreciable difference in size. Fewer associations are present in *E* than in *D*. Ellison believed that the diameter or size of the bivalents may be altered by the general genetical composition of the plant, or as a result of the effect of the environment on the plant. He states (*2, pp. 248–249*):

> The fact that the bivalents in the fertile varieties are of less diameter than in the self sterile ones is difficult to explain. . . . Moreover, since the self sterile varieties are so, either because of their genetical composition or the effect of the environment or both, it is equally feasible that the diameter or size of the bivalents may be altered by the general genetical composition of the plant, or as a result of the effect of environment upon the plant. In either case the effect is the same in that of the cases examined self sterility was associated with an increase in the diameter of the bivalent.

It is quite evident that there is a difference in size between the chromosomes of the F₁ plants, which are mostly pollen-sterile, and the third backcross plants which are mostly pollen-fertile except the chromosomes in figure 3, *H*, which is an unreduced cell.

Figure 3, *G*, has a ring configuration which is seldom seen in this material. The chromosomes are scattered over the spindle, and the prophasic associations appear to have been retained; that is, disjunction has been incomplete. The same is true of figure 3, *N*. What appears to be a quinquevalent is a bi- and a trivalent lying in close proximity. There is a striking contrast in size between the inter-
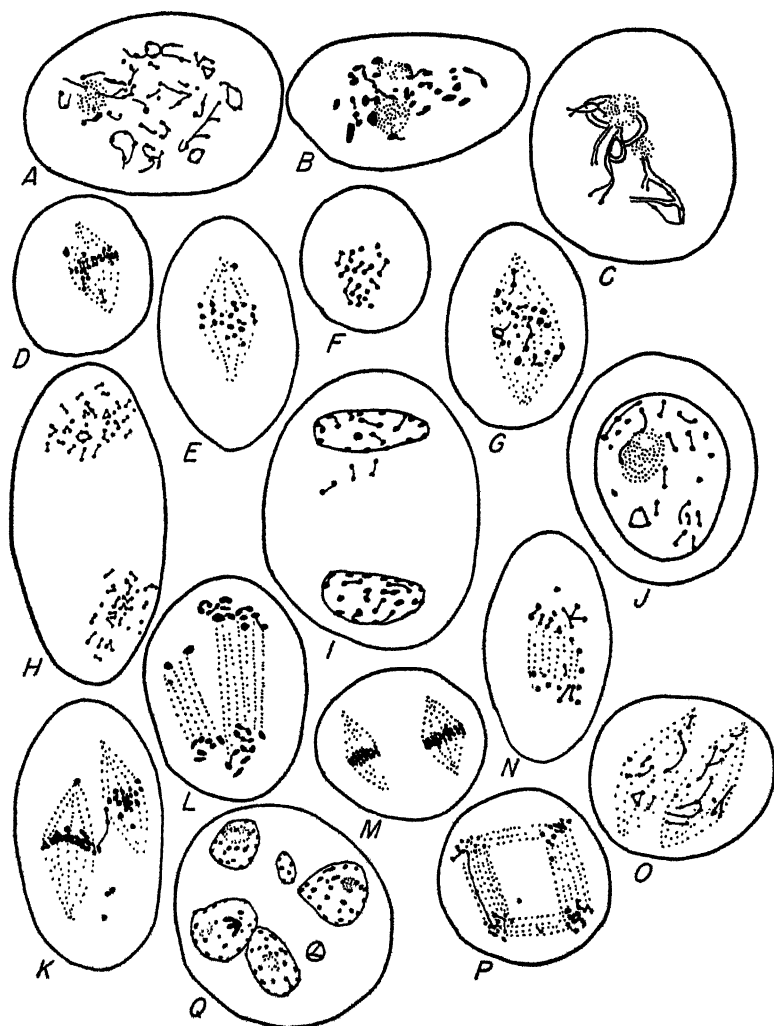
FIGURE 3.—First-generation hybirds: *A*, FEM–1, Early diakinesis; *B*, FEK–1, early diakinesis with two nucleoli; *C*, FEY–1, zygotene with two nucleoli; *D*, FFA–1, irregular first metaphase; *E*, FEJ–1, first metaphase with few associations; *F*, FEJ–1, polar view of metaphase; *G*, FFD–1 first metaphase; *H*, FEL–1, unreduced chromosome groups; *I*, FEQ–1, interkinesis; *J*, FEL–1, unreduced cell; *K*, FEJ–1, irregular second anaphase; *L*, FFC–1, abnormal second anaphase; *M*, FFA–1, regular metaphase; *N*, FEJ–1, irregular first anaphase; *O*, FEL–1, irregular second anaphase; *P*, FEQ–1, chromosome bridges and associations at late anaphase; *Q*, FFA–1 sporocyte with six nuclei.

kinesis figure of *I* and the unreduced chromosome groups in ·*H* (FEL–1). The former cell will form a dyad. In *T* (FEQ–1) three chromosomes have been excluded from the nuclei.

Figure 3, *K* (FEJ–1), *L* (FFC–1), and *O* (FEL–1) are different stages in second anaphase showing various abnormalities. Figure 3, *K* has a trivalent stretched between the two spindles and three univalents in the cytoplasm. One univalent is at the pole in the spindle to the left,

while the other chromosomes are lined up at the plate. Figure 3, *L*, shows a very unequal division with only three chromosomes at the pole in the left-hand spindle. Chromosome associations are present in the spindle at the right. In figure 3, *O*, the chromosomes, with associations present, are scattered over both spindles, with the chromatids greatly elongated. Figure 3, *M* (FFA–1), on the other hand, shows a regular alignment of chromosomes on the metaphase plates.

Figure 3, *P* (FEQ–1), has a chromosome bridge; spindles have formed between all four chromosome groups, while some lagging chromosomes are scattered in the cytoplasm. Figure 3, *Q* (FFA–1), shows six clearly delimited nuclei. The two small nuclei have no nucleoli; one of them has only three chromosomes, still associated. The F_1 pollen is characterized by having a great number of micrograins present. Very seldom does one find a normal quartet of pollen grains; usually five or more are present. It would seem that even when no lagging chromosomes have been included in micrograins the chromosome numbers within the spores are highly irregular. It is, of course, impossible to tell from appearance alone which would be functional.

<div align="center">FIRST BACKCROSS</div>

<div align="center">BREEDING BEHAVIOR</div>

Referring to the general behavior of the F_1 generation upon backcrossing, Reddick (*16, p. 122*) states:

> Instead of selfing first-generation hybrids, pollen of a domestic variety is applied to the stigmas. It is decidedly preferable to use pollen of some variety other than the one used in the first cross. Use of the same variety twice in succession is very likely to result in dwarfing. There are, of course, a great many exceptions. During the past 25 years the writer has grown thousands of first backcross plants. About 12 or 13 per cent of them proved to be susceptible to blight and were discarded. The others when grown in the field gave indications that segregations had occurred. Thus far two individuals have been found which could have been introduced as commercial varieties if their culinary qualities had been satisfactory. All of the others had some or several objectionable characters which precluded their use. The common objections have been—long stolons, extreme lateness, small tubers and deep eyes.

The field notes relating to the first backcross follow:

EKD–1: [*S. demissum* (887)] × [880 (Papa Amarillo)] × [245/52 (U. S. 444/12 × Jubel)]
   "Papa Amarillo" is probably a diploid South American yellow-fleshed variety of *S. tuberosum.* The plant failed to set seed in 1944 and 1946.

EVR–1: [*S. demissum* (178)] × [Ostragis] × [245/52 (U. S. 444/12 × Jubel)]

Fifty-seven immune and 11 susceptible plants were obtained. The plants had a fairly good appearance in the field. They bore small tubers on long stolons. No pollen was effective in seed production in 1946.

EVT–2: [*S. demissum* (178)] × [Ostragis] × [627/226 (Hindenburg × Katahdin)]

Thirty-two immune and 8 susceptible plants were obtained. This plant is Rurallike in appearance; it is a poor cropper, but drought-and frost-tolerant. It failed to set seeds in 1944 and 1946.

FKA–1: [*S. demissum* (178)] × [627/226 (Hindenburg × Katahdin)] × [245/25 (U. S. 444/12 × Jubel)]

Six immune plants and 1 susceptible were obtained. The plants were frost- and drought-tolerant. Two tubers were set on short stolons. In 1944 three pollinations failed; in 1946 one was effective.

FLD–1: [*S. demissum* (431) × [M262/1 (*S. andigenum*) × [627/226 (Hindenburg × Katahdin)]

Seventeen immune and 2 susceptible plants were obtained. Small white tubers are borne on this plant. Pollinations failed in 1945 and 1946.

FQS–1: [*S. demissum* (178)] × [627/226 (Hindenburg × Katahdin)] × [1171 (U. S. 47285)]

Good, open, enormous leaves are formed by this plant in the field. Pollinations failed in the greenhouse.

CYTOLOGY

Figure 4, *B* (FKA–1), *I* (FKD–1), and *J* (EHM–1) are early diakinesis stages showing various associations, including ring and chain formations. Two ring bivalents are seen in *B*. The remaining associations are the same as were found in the F_1 generation; mostly bivalents, trivalents, quadrivalents, and a very few univalents.

Figure 4, *A* (EKA–1), a first division, includes a nucleolus, indicating that an interkinetic nucleus is being formed. The chromosome count is 33.

Figure 4, *C* (EVR–1), is an anaphase in which the associations are very pronounced, and much scattering of the smaller ones has taken place.

Figure 4, *K* (EKD–1), is a diakinesis (prometaphase) figure characteristic of this plant. The chromosomes are very angular and stain deeply. No other plant examined had chromosomes which were so definitely rod-shaped at this stage.

Figure 4, *L* (FLD–1), is an anaphase with a ring configuration as well as a T chromosome in the center right of the figure. T chromosomes are characterized by having a satellite end (*13*). Lamm (*8*) reports their presence in the species of potatoes which he examined. They will be treated further in the discussion. Few were found in this material.

Figure 4, *N* (EHM–1), is a second anaphase which is proceeding irregularly with groups of chromosomes segregating and retaining their connections. Some connections in the spindle to the right have been broken and the dangling ends can be seen.

Figure 4, *O* (EHM–1), is a polar view of a second division. One group has practically all the chromosomes associated, whereas the other group has relatively few.
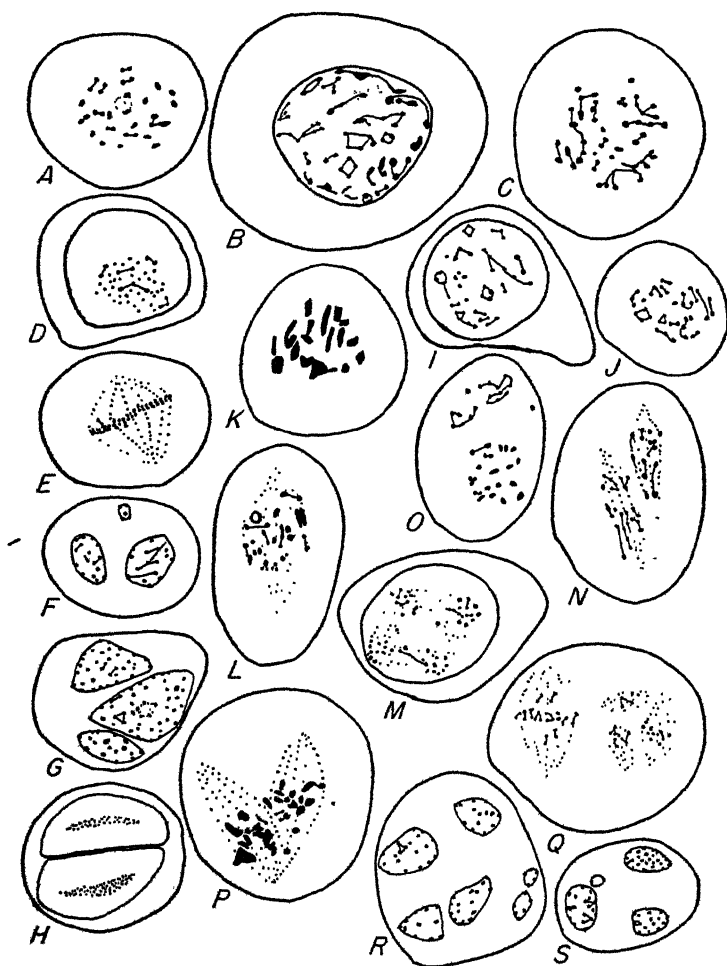
FIGURE 4.—First backcross: *A*, EKA–1, polar view, interkinetic nucleus forming; *B*, FKA–1, early diakinesis; *C*, EVR–1, anaphase with pronounced associations; *D*, CTT–1, polar view of late anaphase (ameiotic division); *E*, CTT–1, metaphase plate (ameiotic division); *F*, CTT–1, interkinetic stage with a micronucleus; *G*, CTT–1, sporocyte with three nuclei; *H*, CTT–1, dyad; *I*, FKD–1, early diakinesis; *J*, EHM–1, early diakinesis; *K*, EKD–1, prometaphase with angular chromosomes; *L*, FLD–1, first anaphase with ring configuration; *M*, EKD–1, late second anaphase with bridges; *N*, EHM–1, irregular second anaphase; *O*, EHM–1, polar view of a second division; *P*, EKD–1, fused second anaphase spindles; *Q*, EIU–2, second anaphase with four spindles; *R*, EIU–2, sporocyte with six nuclei; *S*, FQS–1, sporocyte with three regular nuclei and one micronucleus.

Figure 4, *M* (EKD–1), is a late second anaphase with a bivalent stretched between the lower two nuclei to form a chromosome bridge which has not broken. Association of chromosomes in bi-, tri-, and quadri-valents is seen.

Figure 4, *P* (EKD–1), shows fused spindles with the chromosomes appearing almost as they did at diakinesis.

Figure 4, *Q* (EIU–2), is a second division with three spindles having formed where one would be expected in the right-hand portion of the cell. In the spindle to the left all but four bivalents have lined up on the metaphase plate.

Figure 4, *R* (EIU–2), and *S* (FQS–1) show how irregular the formation of pollen grain nuclei can be. The micronucleus in *S* contains only two chromosomes.

Figure 4, *D-H* are from CTT–1, which had Rural as the female parent. Figure 4, *D* and *E* are stages in ameiotic divisions which will lead to dyad formation. About one-half of the pollen formed is the result of divisions such as these.

Figure 4, *F*, is an interkinesis figure with a micronucleus containing five chromosomes.

Figure 4, *G*, shows three nuclei. Many triads as well as higher grouping of pollen are formed in CTT–1. None of its pollen is functional; in this respect it is like Rural, the female parent. It also resembles Rural in being a functional female. This would seem to indicate that a maternal influence is exerted on the progeny.

SECOND BACKCROSS

BREEDING BEHAVIOR

Regarding the behavior of the second back-cross plants, Reddick (*16, p. 122*) remarks:

If one selects some of the more satisfactory backcrosses and backcrosses again to a domestic variety, a progeny is obtained, with more failures than successes, of which about one-third are susceptible. The plants which do not blight, when grown in the field, have the gross appearance of a field of commercial potatoes. Plants can be found which are mature before the autumnal equinox, and which bear 2, 3, or even 4 pounds of tubers of commercial size. Several thousand selections of plants in this stage have been made and tested. At the present time not one of them has been retained. They have been rejected for various reasons but perhaps the commonest cause of rejection has been deep eyes or irregular contour.

Field notes on the individual plants selected in the present study follow:

EML–1: [*S. demissum* (887)] × [880 (Papa Amarillo)] × [860 (Early Ohio)] × [245/25 (U. S. 444/12 × Jubel)]

The tubers have a pink tinge but are smooth and fleet-eyed. Very few are of commercial size, and the stolons are quite long. Reddick comments that this plant is "still too wild." It failed to set seed in 1944 and 1946.

EWM–3: [*S. demissum* (887)] × [880 (Papa Amarillo)] × [1144 (Metzger's selection)] × [245/25 (U. S. 444/12 × Jubel)]

Thirty-three susceptible and 22 immune plants were obtained from this cross. The immune plants were late and remained green until frost. The tubers were white and 5 of them were of commercial size. Seeds were set in the field and in the greenhouse in 1944, but in 1946

the plant bloomed too late to escape the heat in the greenhouse; the one pollination attempted resulted in failure.

EXW–2: [*S. demissum* (533)] × [860 (Early Ohio)] × [M262/1 (*S. andigenum*)] × [627/226 (Hindenburg × Katahdin)]

Forty-six susceptible and 35 immune plants resulted from this cross. They bore white tubers of noncommercial size and died early. The plant is drought-tolerant. Greenhouse pollinations failed.

EYA–1: [*S. demissum* (533)] × [860 (Early Ohio)] × [M262/1 (*S. andigenum*)] × [627/226 (Hindenburg × Katahdin)]

The plants were late and bore six to eight tubers of commercial size. They set seed very infrequently after pollination.

FSK–1: [*S. demissum* (531)] × [883 (Metzger's selection)] × [245/25 (U. S. 444/12 × Jubel)] × [1065 (Arnica)]

This plant is scab-resistant. In 1946 it set no seed because it was very late in blooming.

FSU–1: [*S. demissum* (741)] × [883 (Metzger's selection)] × [245/25 (U. S. 444/12 × Jubel)] × [DLD–7 (Jubel × F_2 of 883)]

This was one of seven transplants. It bore white, oval, smooth, fleet-eyed tubers of commercial size. Three pollinations produced seed and three failed to do so in 1946.

ESO–1: [Rural] × [*S. demissum* (687)] × [1063 (Ostragis)] × [245/52 (U. S. 444/12 × Jubel)]

Thirty-six immune and 36 susceptible plants were obtained from this cross. The plant is Rurallike and early; it is not frost-tolerant. The tubers have yellow skin and are smooth and double-fleet with a pink dotted eye probably of Ostragis origin.

CYTOLOGY

Figure 5, *A* (EML–1), shows one chiasma at top and perhaps others. It is possible that a piece had been translocated between the chromomeres in the large synaptic configuration in the right of the figure, and that this led to an interrupted pairing. It is difficult to tell whether the point of crossing in the lower configuration is in reality a chiasma or whether the position of the chromosomes is such that it only appears to be, particularly since the chromosomes are definitely unlike in morphology.

Figure 5, *B* (EML–1), is a later stage. Chain associations are prevalent in this nucleus.

Figure 5, *C* (ESO–3), has a predominance of bivalents.

Figure 5, *F*, is a true diakinesis of EML–1. The chromosomes are very large in comparison with those of earlier stages. Few slides with cells in this particular stage were seen. There appear to be only two univalents. The number of chromosomes in the central configuration is impossible to determine.
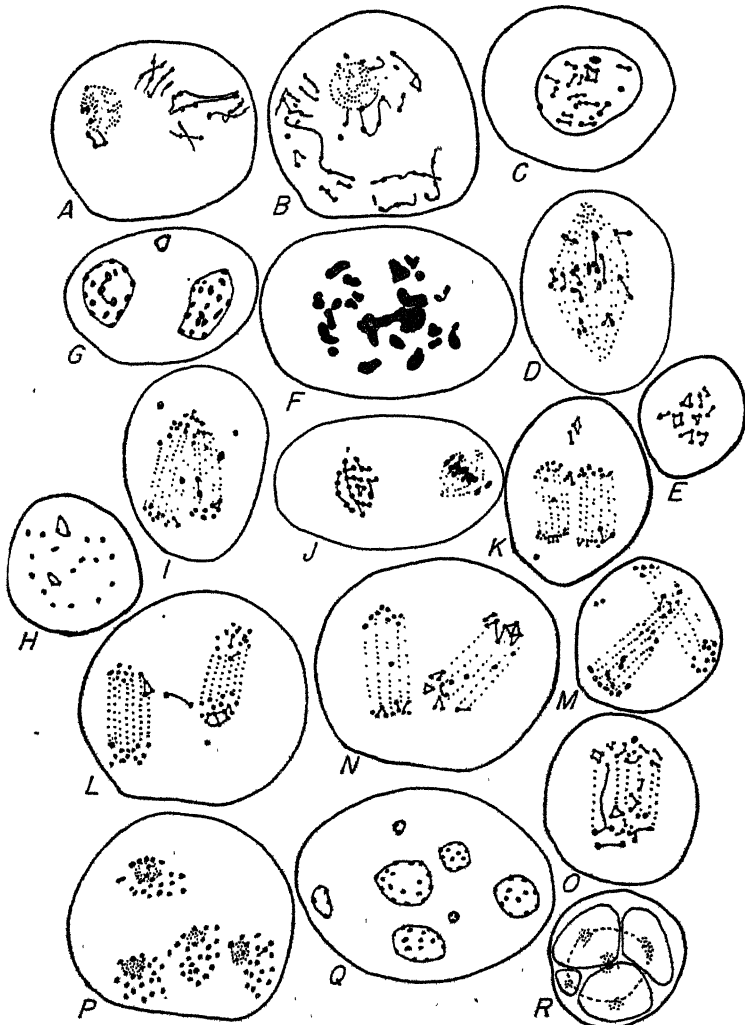
FIGURE 5.—Second backcross: *A*, EML–1, Prophase showing chiasma; *B*, EML–1, early diakinesis; *C*, ESO–3, early diakinesis; *D*, EYA–1, irregular first anaphase; *E*, FSK–1, polar view of first anaphase; *F*, EML–1, true diakinesis; *G*, EWM–3, interkinesis with micronucleus; *H*, FSK–1, polar view of anaphase; *I*, FSU–1, fused second anaphase spindles; *J*, EXW–2, second metaphase; *K*, FSU–1, irregular second anaphase; *L*, ENZ–1, irregular second anaphase; *M*, EWM–3, second anaphase with five groups of chromosomes; *N*, ENZ–1, second anaphase with associated chromosomes; *O*, EWM–3, first anaphase with bridge; *P*, EXW–3, regular nucleus formation; *Q*, EYA–1, irregular nucleus formation; *R*, EYA–1, five spores including a microspore.

Figure 5, *D* (EYA–1), is a first anaphase in which most of the chromosomes are either passing without disjunction to the poles or have never been regularly oriented at the equator.

Figure 5, *E* (FSK–1), is a polar view of an anaphase with bi-, tri-, and quadrivalents present; 24 chromosomes are visible.

Figure 5, *G* (EWM–3), is an interkinesis stage with two regular nuclei and a micronucleus containing three chromosomes. This plant was self-fertile.

Figure 5, *H* (FSK–1), is a polar view of an anaphase group in which 22 chromosomes are present, all of them separate except 2 rings of 3 chromosomes.

Figure 5, *I* (FSU–1), shows fusing second anaphase spindles. Several chromosomes are entirely excluded from the figure and some are lagging on the spindle.

Figure 5, *J* (EXW–2), is a second metaphase with the commonly found orientation of the spindles. An excessive amount of association seems to be present in the polar view. The side view seems normal except for two chromosomes at the edge of the spindle.

Figure 5, *K* (FSU–1), shows a second anaphase with six chromosomes at the top of the cell completely out of the spindle area, and one at the lower left of the spindles. On the spindle are lagging univalents and between the spindles is a bivalent. The trivalent in the lower group of the right-hand spindle, because of the size of the chromosomes, gives the impression of having gone through both meiotic divisions intact.

Figure 5, *L*, is another anaphase with a bivalent stretched between the two spindles; an excluded univalent lies below one spindle. The chromosomes are associated in a network in the lower portion of the spindle to the right. Such networks were infrequently encountered and *L* and *N* were included to illustrate their occurrence. That they are artifacts is a possibility, but it would seem untenable to assume that the fixation is improper in these two isolated cases and proper for the remaining chromosomes in the cells. In the upper portion of the spindle to the left in *L* is a quadrivalent.

Figure 5, *N* (ENZ–1), shows a network of eight chromosomes as well as lagging univalents.

Figure 5, *M* (EWM–3), with five groups of chromosomes on two spindles is rather difficult to explain. Two chromosomes have been excluded in the left-hand portion of the cell.

Figures 5, *P* (EXW–3), *Q* (EYA–1), and *R* (EYA–1) are stages in the delimination of pollen nuclei and grains. Two micronuclei, each containing three chromosomes, as well as five fairly large nuclei may be seen in *Q*. Such groups of four pollen grains and one micrograin are very commonly found in the anthers of second backcross individuals.

THIRD BACKCROSS

BREEDING BEHAVIOR

In regard to the third backcross Reddick (*16, p. 122*) says:

Since a high proportion of the plants in the second backcross still carry the gene for immunity, a third backcrossing has been tried. About one-half of the progeny continues to give immune reaction by inoculation and in this generation many more desirable types can be found and tests of these, which are now in progress, indicate that suitable types have been isolated which have enough of the desirable horticultural characteristics to make them satisfactory commercial varieties.

Field notes on the plants examined in the present study follow:

CRH–7: [Rural] × [*S. demissum* (687)] × [103/14 (F₂, Rural × Steinthaler)] × [851 (Katahdin)] × [860 (Early Ohio)]

This is the blight-resistant Empire which is soon to be released. From 50 seeds 16 plants were obtained in 1940, of which CRH-7 was a selection. It is a large green plant which bears four to six commercial tubers. The pollen is fair, and in 1946 the plant set seed from three pollinations and failed to set seed from two. It is medium in time of maturity; by the first of October the plants are dead.

DGH–7: [*S. demissum* (687)] × [103/14 (F₂, Rural × Steinthaler)] × [851 (Katahdin)] × [860 (Early Ohio)] × [528/274 (Jubel × U. S. 44537)]

There were 178 susceptible and 90 immune plants obtained from this cross of which DGH-7 was a selection. It has a rugose leaflet and is an excellent plant, medium in time of maturity. It bears many tubers of commercial size which are mealy and good. This plant failed five pollinations in 1946.

EOJ–1: [*S. demissum* (687)] × [103/14 (F₂, Rural × Steinthaler)] × [890 (*S. andigenum*)] × [M166 (*S. andigenum*)] × [1064 (Hindenburg)]

Thirty-six transplants were made from 50 seeds. The plants were late and bore about 7 tubers of commercial size and 8 the size of eggs. The tubers have pink-dotted eyes, which in all probability is due to the influence of 890. No flowers were borne in 1944 or 1946.

FIX–1: [*S. demissum* (687)] × [103/14 (F₂, Rural × Steinthaler)] × [860 (Early Ohio)] × [M262/1 (*S. andigenum*)] × [245/25 (U. S. 444/12 × Jubel)]

Thirty-one susceptible and 11 immune plants were obtained. These plants stayed green until frost and were drought-tolerant. Seeds were set natuarally in the field. In the greenhouse in 1945 this plant set seeds after 3 pollinations and failed to set seeds after 3. It was late in blooming in 1946 and the 1 pollination effected failed to set seeds. The plant is much too late for commercial purposes, although it bore 1 tuber of commercial size and 5 the size of eggs.

FMP–1: [*S. demissum* (533)] × [860 (Early Ohio)] × [1067 (Hindenburg × Centifolia)] × [OE/1 (F₂ of Metzger's seedling 883)] × [245/25 (U. S. 444/12 × Jubel)]

Fourteen susceptible and 25 immune plants were obtained. FMP-1 set seeds in 1944 in the field. It is drought-tolerant and scab-resistant. Seven pollinations failed and one succeeded in 1946. The tubers are largely noncommercial.

CYTOLOGY

Figure 6, *A* (CRH-7), is an early diakinesis stage with uni-, bi-, tri-, and quadrivalents.

Figure 6, *B* (DGH-7), is a polar view of a first metaphase in which 24 chromosomes can be distinguished. One association of 5 and 6 associations of 2 chromosomes are seen.

two tubers of commercial size and three the size of walnuts.   No seeds were set in the greenhouse in 1946.

FQE–2: [*S. demissum* (687)] × [103/14 (F$_2$, Rural × Steinthaler)] × [851 (Katahdin)] × [860 (Early Ohio)] × [528/118 (Jubel × U. S. 44537)] × [1067 (Hindenburg × Centrifolia)]

This plant was one of 21 transplanted.   It is erect and of excellent size in the field.   The tubers are white, oval, smooth, and fleet-eyed; 1 the size of an egg and 6 the size of walnuts were obtained in 1945. No seeds were set in the greenhouse in 1946.
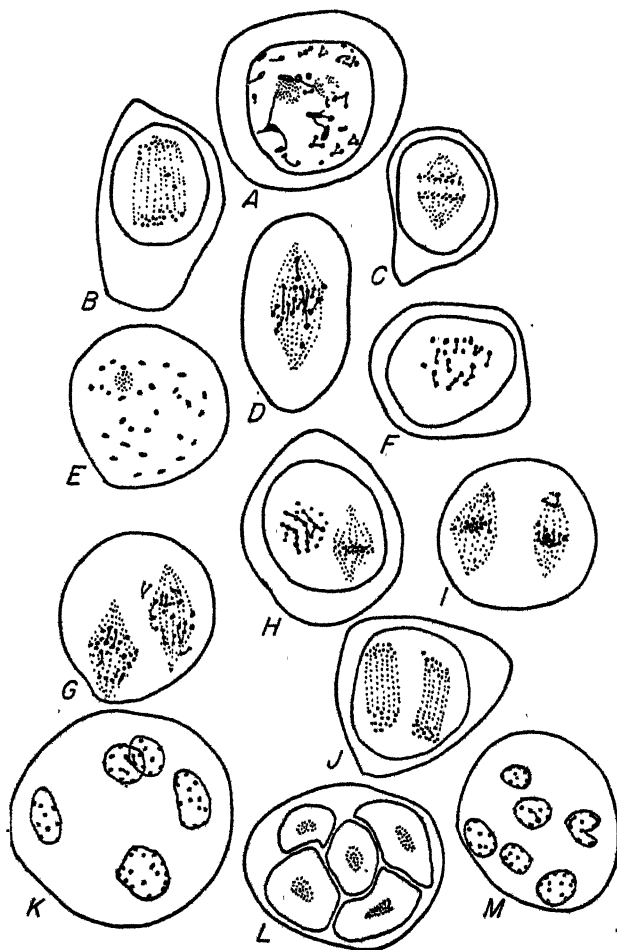
FIGURE 7.—Fourth backcross: *A*, FQE–1, early diakinesis with two nucleoli; *B*, FAU–1, late first anaphase; *C*, FAU–1, regular first anaphase; *D*, FAU–1, first anaphase with associations; *E*, FQE–1, polar view of metaphase; *F*, FNH–4, polar view of first metaphase; *G*, FQE–1, irregular second anaphase; *H*, FAU–1, second metaphase; *I*, FNH–4, irregular second metaphase; *J*, FAU–1, regular second anaphase; *K*, FQE–1, sporocyte with five nuclei; *L*, FNH–4, five spores formed; *M*, FNH–4, six nuclei in sporocyte.

Figure 7, *A*, is an early diakinesis of FQE–1. Two nucleoli are present, with most of the chromosomes appearing as uni-, bi-, and trivalents.

Figures 7, *B* (FAU–1) and *C* (FAU–1) show regular first anaphases, although two chromosomes are lagging in *B*. The absence of associations and the small size of the chromosomes are strikingly evident.

Figure 7, *D* (FAU–1), shows the irregular character of some of the anaphase figures in this same plant. Here are all of the characteristics of the earlier backcrosses.

Figure 7, *E* (FQE–1), is a polar view of a first metaphase in which 30 chromosomes can be counted. No associations are present, and the number is that expected in an F_1 individual.

Figure 7, *F* (FNH–4), is a polar view in which 26 chromosomes, many of them associated, are seen.

Figure 7, G (FQE–1), shows the customary irregular behavior, with associations retained but not in such high numbers as were found in the earlier backcrosses. A trivalent and a bivalent are excluded from the spindle at the right.

Figure 7, *H* (FAU–1), and figure *I* (FNH–4) are of the second division. The polar view at the left in *H* has a high number of associated chromosomes. The chromosomes involved appear to be larger than the unassociated ones. The metaphase figure on the right seems to be proceeding regularly with no associations present. Both spindles in *I* have chromosomes out of line and associations present.

Figure 7, *J* (FAU–1), is a late second anaphase which is proceeding regularly.

Figures 7, *K* (FQE–1), *L* (FNH–4), and *M* (FNH–4) show supernumerary nuclei and pollen grains which result. Normal quartets, however, are quite prevalent in this group of plants.

DISCUSSION

The phenomenon which is most notable in this study of the backcross progeny of a cross between a hexaploid and a tetraploid plant is the continued association of chromosomes found throughout the various stages of meiosis, particularly in the anaphase spindles. In the wild species of *Solanum* with fertile pollen this association is not found, but in the varieties of *S. tuberosum* examined, and in all the hybrids studied, retained anaphase associations and sterility of pollen seem to be directly proportional. If, indeed, in these plants, groups of chromosomes segregate intact, as would appear from the foregoing illustrations, then the number of chromosomes present would give no indication of the ability or inability of the pollen grains to function; it would rather be a matter of which chromosomes are present that would determine functional capacity. Lamm (*8, p. 57*) states: • "A comparison between the percentage of balanced II–M plates and the percentage of good pollen shows that some of the apparently good pollen must have an unbalanced chromosome number."

Becker (*1, p. 28*) investigated the chromosome counts of 15 F_2

plants of a *S. demissum* x *S. tuberosum* cross and made the following statement concerning them:

There was no apparent relationship between chromosome number and either tuber formation or seed production. The approximate chromosome counts of those that set seed as well as tubers were 49, 50, 51, and 56; of those forming only tubers but no seed 50, 53, 54, 56, and 58; and of those forming neither tubers nor seed 48, 52, 53, 54, 56, and 58.

This factor would further explain the seemingly inconsistent behavior of plants from year to year where fertility is concerned. In a pollen-sterile plant some functional grains could be produced, although the odds in its favor would be negligible. Longley and Clark (*11, p. 882*), in their study of chromosome behavior and pollen production in the potato, made the following statement:

It seems apparent that in different varieties of potatoes the chromosome complement does not show the harmony observed in wild species . . . So pronounced is it [this lack of harmony] in a large percentage of the varieties that there is little or no normal pollen mother-cell development . . . In a very few varieties the chromosome complement seems to be in harmony and the meiotic behavior is almost as regular as that found in material from wild species.

They further state (*p. 883*):

Although environment may modify somewhat the chromosome behavior during the reduction divisions of the developing pollen mother cells, the writers agree with Fukuda (*5*) [7] that the lack of harmony of the chromosome complement of tetraploid varieties of potatoes is the major cause for the failure to produce, in potatoes, normal 4-cell polen tetrads.

Carrying the situation a bit further, the present writer is of the opinion that the lack of harmony between the chromosome complement and the cytoplasm results in the formation of new connections or in the retention of synaptic associations, which remain intact throughout the divisions, this in turn causing a final unbalance within the chromosome complements in many if not all of the pollen grains. Regardless of regular chromosome numbers and quartet formation, such pollen is functionless. It is, of course, recognized that under these circumstances regular chromosome numbers and quartet formation do not preclude functionless pollen. Becker (*1, p. 27*) found that, although the percentage of stainable pollen for *S. demissum* was 61.3 percent and the mean for the F_1 hybrids was 59.3 percent, the germinated grains in *S. demissum* constituted 26.8 percent, whereas in the hybrids only 2.3 percent germinated. What percentage of these would finally have achieved fertilization in actual tests is not known, but in all probability the percentage would have been lower, judging by the experience of the author and of Reddick,[8] who found that an entire summer spent in hand-pollination of F_1 hybrids of *S. demissum* and *S. tuberosum* resulted in the production of only 98 seeds.

Considering the heterozygous nature of the *S. tuberosum* varieties, a wide range of variation would be expected in the progeny, but such was not the case; most of the plants resembled the *S. demissum* parent, when *S. demissum* was used as the female, and continued to do so in following generations. Obviously the *S. demissum* complement was in rare cases being recovered in both pollen grains and megaspores. Considering the pairing behavior in the F_1 plants investi-

[7] See Literature Cited, reference (*4*), of the present paper.
[8] Oral communication.

gated, wherein synaptic associations of the kinds common to both *S. demissum* and *S. tuberosum* are formed, with no consistent formation of 24 bivalents and 12 univalents observed, a possible explanation may be that pairing inter se of chromosomes derived from each of the parental individuals takes place; that is, that autosyndesis of the *demissum* chromosomes and of the *tuberosum* chromosomes occurs. If such were the case, the *S. demissum* chromosomes functioning in the *S. demissum* cytoplasm would infrequently give rise to a functional grain when by chance they were included in a balanced complement.

This is contradictory to the findings of Salaman (*17; pp. 1238–1239*), who states:

At the reduction division of the F_1 24 domestic chromosomes units with 24 *S. utile* [*demissum*] chromosomes giving 24 bivalents, the other 12 *S. utile* chromosomes remain unpaired and segregate at random in the heterotype and homotype divisions so that the composition of the pollen-grains varies from 26 to 32. On the assumption that the same type of segregation occurs in the megaspore, mating of gametes would give a variety of forms possessing 24 bivalents + a varying number of univalents. Chance mating of gametes will give two types of chromosome complex in the F_2:

1. Those containing 60 to 72, i. e., plants derived from gametes containing 6 or more than 6 univalents. . . .
2. Those containing from 48 to 60, i. e., derived from gametes containing less than 6 univalents. . . .

The extra 12 univalents in the F_1 are *S. utile* chromosomes; when 6 or more of them enter the F_2 zygote the individual is *S. utile*-like, in fact it may be suggested that certain of the *S. utile* chromosomes are very stable and that there may be a tendency for gametes possessing many of these extra *S. utile* chromosomes to unite. A pure *S. utile* type of plant is soon extracted from the cross (e. g. in F_2), and the F_4 gives a majority of gametes containing 36 chromosomes (i. e. containing the extra set of *S. utile* chromosomes). The fact that these apparently pure looking *S. utile* plants contain some domestic blood is revealed by the fact that when back-crossed to the parent domestic forms predominantly domestic plants . . . reappear. There is also evidence from the Mendelising of parental characters that the 24 *S. utile* chromosomes and the 24 domestic chromosomes which form bivalents segregate normally, and must not be thought of as segregating in sets of domestic or sets of *S. utile*.

Unfortunately, no illustrations of the cytological features upon which Salaman based his conclusions have been published, and in the material which is here presented, no such regularity as that described by Salaman has been found. There is one initial difference in the material investigated: Salaman used as the pollen parent a "highly inbred domestic seedling," which he says (*17, p. 1236*)—

has the haploid number 24. . . . The chromosomes are similar in size and shape to those of *S. utile*. The cytology is slightly aberrant, but, compared with the behaviour in some of the domestic varieties examined, it might be characterized as practically normal. Tetrad formation proceeds normally but there is a certain amount of sterile pollen.

There is evidence that in *Solanum* hybrids pairing of chromosomes within a parental complement takes place. Ellison (*3*) reports that in the cross *S. nigrum* ($n \times 36$) = *S. nitidibaccatum* ($n \times 12$), although bivalents were formed and the divisions were very regular, there was complete sterility. The majority of the pollen grains appeared to be quite normal, but he states (*3, p. 477*): "While normal pollen grains were formed containing 24 chromosomes and the anthers dehisced, the plant failed to set any viable seed under self-pollinating conditions."

In the opinion of the writer there is no way to resolve the dif-

ference of opinion regarding the pairing procedure in *S. demissum* ×
S. tuberosum hybrids except by means of visible markers on the
chromosomes that can be followed through synapsis, as has been done
in maize.

Secondary associations in the material presented have not been
stressed, since there seems to be a difference of opinion concerning
the phenomenon (*8, 9, 5*). The associations mentioned in this study
refer to actual physical connections and not proximity in position of
chromosomes. There is some question as to whether these physical
extensions are chromatids that remain unbroken throughout the
divisions, or whether, because of "stickiness" of the chromosomes,
they reunite at a previous breakage point. In part, the behavior of
the hybrids' chromosomes described in this paper are reminiscent of
the behavior of the asynaptic diploid and tetraploid plants derived
from a colchicine-treated *S. rybinii* chimera described by Lamm (*8*),
particularly in the bridge formation and lagging chromosomes.
There is some indication that nucleic acid starvation causes stickiness
in chromosomes. Lamm (*8, p. 33*) makes the following statement:

. . . in asynaptic *S. Rybinii* a gene mutation may be responsible for nucleic-acid
starvation. At I—M the bridges without fragments could then be of two cate-
gories. One of these has already been described, namely, bridge formation due
to arrested terminalization in gemini. The other category is thought to be due
to sticking univalents. At II—A sister chromatid reunion might be responsible
for most of the bridges, this reunion in turn being probably caused by disturbed
nucleic-acid metabolism.

This might also be the explanation of the extensions between the
chromosomes in some cases where it is quite certain that they were
not retained during the divisions.

That the connections are artifacts is in the writer's opinion un-
tenable, since they have been found by cytological investigators in
other genera of solanaceous plants (*20*). The regularity with which
they are found in spindles of pollen-sterile plants with a correspond-
ing lack in pollen-fertile plants, all of which were given the same
treatment, would indicate that they have some significance and are
not to be attributed to improper fixation.

In the hybrids discussed in this paper, the stickiness would in all
probability not be due to a gene but to the malfunctioning of the
chromosomes in the cytoplasm, which might very well result in a
disturbance of the nucleic acid metabolism. Reciprocal crosses be-
have differently in these hybrids, the only unfortunate circumstance
being that sterility factors are so prevalent in the Rural varieties
that no adequate comparison can be made. Lamm (*7, p. 207*) makes
this statement concerning the behavior of reciprocal crosses: "This
dissimilar chromosome mechanism in the P. M. C.'s of these *Solanum*
hybrids after reciprocal crossings may be due either to cytoplasmic
inheritance or to maternal effects."

The T chromosome phenomenon mentioned in the cytological ac-
count has been found in *Solanum* species by Lamm (*8*). Prakken
and Müntzing (*13, p. 480*) have reported this meiotic peculiarity in
rye, which they attribute to "an interaction between the genotypical
constitution of the various lines and a structural difference separating
the lines with and without a T chromosome pair." The behavior of
the T chromosomes simulates the presence of a terminal centromere

in their estimation, since in rye the satellitelike ends of the chromosomes are oriented toward the poles. In potatoes, however, this is not the case, as is pointed out by Lamm (*3, p. 31*):

As a rule the terminal satellite-like bodies were orientated towards the equator during the first division. In the diploid, out of 64 counted univalents with such bodies, 61 were orientated towards the equator.

The T arm of the chromosome in figure 4, *L*, of this paper is likewise oriented toward the equator.

Taking into consideration all of the abnormalities hitherto outlined, it would seem that the breeding of blight-proof potato varieties by using *S. demissum*×*S. tuberosum* hybrids must remain dependent upon the discretion of the breeder in effecting crosses and then selecting for the characters desired, with little hope of establishing a breeding program wherein expectations, genetically speaking, may be realized. It is very fortunate that any plant having the desired characters can be reproduced asexually in the potato, since in view of the faulty sexual mechanism in the hybrids, it would be impossible to duplicate the specific set of characters from seed.

SUMMARY AND CONCLUSION

The meiotic divisions in the microsporocytes of *Solanum demissum*, *S. tuberosum*, and their hybrid progeny through four backcrosses to *S. tuberosum* have been studied.

Irregularities of meiotic behavior in the hybrids include lagging chromosomes, spindle abnormalities, high multivalent formation, delimitation of fewer or more than four spores, and pollen sterility in most cases. In the divisions resulting in pollen sterility, groups of chromosomes retaining their primary synaptic associations are visible on both anaphase spindles, while in normal polyploid plants segregation of chromosomes in multivalents is at random. Nucleic acid starvation due to the malfunctioning of the chromosomes in the cytoplasm is a possible reason for the retention of associations and bridge formation. These irregularities are found throughout the first-generation hybrids and subsequent backcrosses, but a tendency toward more regularity and greater fertility is expressed in the third and fourth backcrosses. However, the divisions of the third and fourth backcrosses are still highly irregular. and the plants retain many of the undesirable characters of *S. demissum* as well as chromosome numbers which approximate those of the F_1 hybrids. It is suggested that autosyndesis of *S. demissum* and of *S. tuberosum* chromosomes occurs.

The irregularities observed are shown not to be the result of environmental conditions, but to have their origin in constitutional factors involving both the chromosome complement and the cytoplasm.

It is concluded that breeding for blight resistance in commercial varieties of *S. tuberosum* by using *S. demissum*×*S. tuberosum* crosses must remain primarily a trial and error method.

LITERATURE CITED

(1) BECKER, C. L.
    1939. INHERITANCE STUDIES IN THE INTERSPECIFIC CROSS SOLANUM DEMISSUM LINDL. × S. TUBEROSUM L. Jour. Agr. Res. 59: 23–39.

(2) ELLISON, W.
1936. MEIOSIS AND FERTILITY IN CERTAIN BRITISH VARIETIES OF THE
CULTIVATED POTATO (SOLANUM TUBEROSUM L.). Genetica 18:
217–254, illus.
(3) ――――
1936. SYNAPSIS AND STERILITY IN A SOLANUM HYBRID. Jour. Genet. 32:
[473]–477, illus.
(4) FUKUDA, Y.
1927. CYTOLOGICAL STUDIES ON THE DEVELOPMENT OF THE POLLEN-
GRAIN IN DIFFERENT RACES OF SOLANUM TUBEROSUM L., WITH
SPECIAL REFERENCE TO STERILITY. Bot. Mag. [Tokyo] 41:
[459]–476, illus.
(5) HEILBORN, O.
1936. THE MECHANICS OF SO-CALLED SECONDARY ASSOCIATION BETWEEN
CHROMOSOMES. Hereditas 22: [167]–188, illus.
(6) JORGENSEN, C. A.
1928. THE EXPERIMENTAL FORMATION OF HETEROPLOID PLANTS IN THE
GENUS SOLANUM. Jour. Genet. 19: [133]–211, illus.
(7) LAMM, R.
1941. VARYING CYTOLOGICAL BEHAVIOUR IN RECIPROCAL SOLANUM
CROSSES. Hereditas 27: [202]–208, illus.
(8) ――――
1945. CYTOGENETIC STUDIES IN SOLANUM, SECT. TUBERARIUM. Hereditas
31: 1–128, illus.
(9) LAWRENCE, W. J. C.
1931. THE SECONDARY ASSOCIATION OF CHROMOSOMES. Cytologia 2: 352–
384, illus.
(10) LINDSEY, J.
1848. NOTES ON THE WILD POTATO. Hort. Soc. London Jour. 3: 65–72,
illus.
(11) LONGLEY, A. E., and CLARK, C. F.
1930. CHROMOSOME BEHAVIOR AND POLLEN PRODUCTION IN THE POTATO.
Jour. Agr. Res. 41: 867–888, illus.
(12) MÜNTZING, A.
1933. STUDIES ON MEIOSIS IN DIPLOID AND TRIPLOID SOLANUM TUBEROSUM
L. Hereditas 17: [223]–245, illus.
(13) PRAKKEN, R., and MÜNTZING, A.
1942. A MEIOTIC PECULIARITY IN RYE, SIMULATING A TERMINAL CENTRO-
MERE. Hereditas 28: [441]–482, illus.
(14) PROPACH, H.
1938. CYTOGENETISCHE UNTERSUCHUNGEN IN DER GATTUNG SOLANUM,
SECT. TUBERARIUM. IV. TETRAPLOIDE UND SESQUIDIPLOIDE
ARTBASTARDE. Ztschr. f. Induktive Abstam. u. Vererbungslehre
74: [376]–387, illus.
(15) REDDICK D.
1934. ELIMINATION OF POTATO LATE BLIGHT FROM NORTH AMERICA.
(Phytopath. note) Phytopathology 24: 555–557.
(16) ――――
1943. DEVELOPMENT OF BLIGHT-IMMUNE VARIETIES. Amer. Potato Jour.
20: 118–126.
(17) SALAMAN, R. N.
1928. ABNORMAL SEGREGATION IN FAMILIES ARISING FROM THE CROSS
SOLANUM UTILE × SOLANUM TUBEROSUM. Internatl. Genet.
Cong. [Berlin] 1927: [1230]–1239, illus.
(18) SMITH, H. B.
1927. CHROMOSOME COUNTS IN THE VARIETIES OF SOLANUM TUBEROSUM
AND ALLIED WILD SPECIES. Genetics. 12: [84]–92, illus.
(19) STOW, I.
1927. A CYTOLOGICAL STUDY ON POLLEN STERILITY IN SOLANUM TUBER-
OSUM L. Jap. Jour. Bot. 3: [217]–238, illus.
(20) UPCOTT, M.
1935. THE CYTOLOGY OF TRIPLOID AND TETRAPLOID LYCOPERSICUM
ESCULENTUM. Jour. Genet. 31: 1–19, illus.

COMPOSITION OF SHARK MEAL [1]

By SIDNEY P. MARSHALL, *associate dairy husbandman,* and GEORGE K. DAVIS, *nutrition technologist, Department of Animal Industry, Florida Agricultural Experiment Station* [2]

INTRODUCTION

Shark meal, a livestock feedstuff high in crude protein content, has been produced in Florida and along the Pacific seaboard in increasing quantities during the past 7 years. This product has proved to be an excellent protein supplement in rations for growing dairy calves (*12*),[3] chicks (*13*), and growing-fattening swine (*14*). Analyses previously reported for shark meal (table 1) show it to be high in crude protein, calcium, and phosphorus. Assays for all of the constituents commonly determined in routine feedstuff analyses have not been reported for any single sample of shark meal.

French [4] stated that shark meal contains some urea nitrogen, which, when calculated as crude protein ($N \times 6.25$), gives a value for the nitrogenous constituents that is excessive. Rhian, Carver, Harrison, and Hamm (*16*) found from 0.09 to 4.37 percent of urea in eight samples of meal made from dogfish sharks. Analyses of these samples by a procedure for determing urea and ammoniacal nitrogen (*3, p. 79*), but omitting the urease, yielded what Rhian et al. termed "nitrogen fraction B" in amounts ranging from 0.35 to 3.55 percent.

Shipments of shark meal received in paraffined containers and paper parcels at the Florida Agricultural Experiment Station had a strong fishy odor in which it was thought that ammonia could be detected. Any loss of nitrogen as ammonia would indicate the presence of it or a precursor in the meal. To acquire more comprehensive information on the composition of shark meal, routine feedstuff analyses were made on 19 samples and spectrographic analyses for the presence of 33 elements were made on the ash of 12. A study was also made of the nature and amounts of nonprotein nitrogen present, and samples were assayed for urease activity.

Journal of Agricultural Research,   Vol. 76, Nos. 9, 10
Washington, D. C.   May 1, 15, 1948
Key No. Fla.–24

(213)

TABLE 1.—*Composition of shark-meal samples as reported by different investigators*

Investigator	Processing method	Kind of shark	Analyses							
			Water	Crude protein	Ether extract	Crude fiber	Ash	Ca	P	Chlorine
			Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Rhian, Carver, Harrison, and Hamm (16).	Wet	Dogfish	5.0	67.3	20.8	------	------	3.14	2.01	------
	---do---	---do---	4.0	67.2	21.8	------	10.0	2.29	1.77	------
	Dry	---do---	5.0	77.3	13.3	------	13.3	2.33	1.87	------
	---do---	---do---	11.0	78.5	8.4	------	11.1	1.76	1.52	------
	---do---	---do---	10.5	77.2	7.1	------	11.4	1.94	1.75	------
Evans, Carver, and Hamm (5).	Wet	---do---	3.7	70.8	17.9	------	9.1	2.30	1.62	------
	---do---	---do---	4.5	73.2	17.4	------	9.9	2.52	1.73	------
	---do---	---do---	3.4	70.2	20.8	------	9.7	2.38	1.69	------
	Dry	---do---	7.5	82.1	10.5	------	11.0	2.15	1.74	------
French [1]	Wet	Unknown	10.6	82.4	6.3	1.2	10.1	------	1.50	0.46
	---do---	---do---	4.8	85.7	.9	.9	14.2	------	------	------
	---do---	---do---	8.4	85.6	1.0	1.0	13.3	------	------	.03
	---do---	---do---	5.1	87.0	1.2	1.8	10.1	------	------	------
	---do---	---do---	7.6	78.9	1.4	.9	11.0	------	------	------
	---do---	---do---	8.3	80.6	------	------	------	------	------	------
Almquist (1)	Unknown	---do---	------	74.0	------	------	------	------	------	------
	---do---	---do---	------	81.1	------	------	------	------	------	------
Grau and Almquist (7)	---do---	---do---	------	64.3	------	------	------	------	------	------

[1] French, R. M. Private communication to R. B. Becker, 1944.

MATERIAL AND METHODS

Nineteen samples of commercial shark meal manufactured by a wet-processing method (14) from sharks caught off the Florida coast during January, February, and March were available for analyses. Moisture, crude protein (N X 6.25), ether extract, crude fiber, ash, and calcium were determined according to the A. O. A. C. (2) methods for grain and stock-feed analyses. The phosphorus was determined by the method of Fiske and Subbarow (6). Magnesium was determined essentially by the method of Handy (10) except that HCl was used and bromcresol green replaced methyl red as the indicator. Nitrogen-free extract was calculated according to the method described by Morrison (15). Seventeen of these samples were analyzed for urea by Griem's modification (8) of the Griem and Walker method (9). Check determinations omitting the urease also were made simultaneously on these samples and the nitrogen values obtained are designated herein as nitrogen fraction A.

Twelve samples of shark meal were analyzed for the presence of 33 elements by the rough estimate method (17), using a Littrow type spectrograph. These samples were prepared for analysis by weighing 6 gm. of shark meal into a glazed silica evaporating dish and ashing at temperatures not exceeding 450° C. in a muffle furnace with concealed heating elements. The silica dishes had been leached previously for 24 hours in a mixture of 1 part of HCl, 1 part of HNO₃, and 4 parts of water which had been redistilled in a pyrex glass apparatus. The ash of each sample was placed in a glass bottle, which was sealed with a paper-lined screw top, and transferred to the spectrographic laboratory for analysis.

Tests were made for urease activity on five samples of shark meal. The apparatus used was essentially as described by Hawk and Bergeim (11).

Two grams of shark meal, 0.1 gm. of urea, 10 ml. of Clark's (*4*) phosphate buffer solution (pH 6.8), and 150 ml. of water that had been redistilled in a pyrex glass apparatus were placed in a large cylinder which then was stoppered tightly. These materials had been stored previously at a temperature of 40°±1° C. and, after mixing, were incubated at this temperature for 1 hour. Air then was aspirated through this material into 0.1 N HCl for 15 minutes and the excess acid titrated with 0.1 N NaOH. A blank determination with urea omitted was run simultaneously with the assay of each sample.

To study certain changes which might be associated with a loss of nitrogen by volatilization, nitrogen fraction A, urea, pH (using a glass electrode), and moisture were determined on five samples within a few days after processing and again after exposure to the atmosphere for 70 days of storage. The samples were prepared for storage by placing approximately 150 gm. of shark meal in a 400-ml. pyrex glass beaker which then was covered with eight thicknesses of cheesecloth, and set in a room where contamination would be avoided.

EXPERIMENTAL RESULTS

The averages of the analyses for 19 samples of shark meal were: Moisture 9.18 percent, crude protein 78.07, ether extract 2.80, crude fiber 0.32, ash 13.97, calcium 3.48, phosphorus 1.92, and magnesium 0.17 percent. Since the sum of the ingredients for which analyses were made exceeded 100 percent in most cases, calculation of the nitrogen-free extract by difference gave values for only 3 of the samples. Seventeen of these samples contained an average of 1.75 percent of urea and 0.49 percent of nitrogen fraction A. Analyses of each sample together with average and standard error (*18*) are presented in table 2.

TABLE 2.—*Composition of shark-meal samples with average and standard error*

Sample No.	Water	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Ca	P	Mg	Urea	Nitrogen fraction A
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	5.80	83.06	0.12	0.28	1.34	9.40	2.42	1.69	0.16		
2	10.94	82.56	1.58	.37	0	11.66	2.90	1.52	.27		
3	14.95	70.85	4.21	.32	0	13.24	3.19	1.76	.22	1.72	0.490
4	15.34	63.25	3.96	.20	0	19.03	5.24	2.92	.33	1.05	.765
5	14.53	66.04	4.02	.33	0	17.93	4.97	2.60	.25	1.32	.625
6	14.55	68.35	4.09	.19	0	16.46	4.39	2.48	.28	1.48	.680
7	13.83	80.90	4.70	.26	0	16.03	4.33	2.40	.28	1.44	.670
8	7.23	82.12	2.19	.43	0	15.75	3.19	1.72	.13	3.35	.325
9	9.91	87.48	2.78	.17	0	11.28	2.52	1.50	.15	1.25	.465
10	10.04	70.35	4.19	.22	3.49	11.71	2.84	1.60	.20	1.85	.405
11	3.14	79.94	6.44	.20	0	13.76	3.10	1.72	.19	2.51	.365
12	7.58	82.02	2.22	.30	0	12.80	2.57	1.52	.19	.94	.410
13	11.23	77.44	.98	.50	0	14.69	3.89	1.80	.07	2.06	.460
14	4.77	85.50	1.64	.26	0	13.86	3.09	1.76	.06	2.49	.445
15	7.66	82.50	1.39	.57	0	11.97	2.79	1.52	.04	3.16	.270
16	6.36	78.69	.94	.32	0	15.08	3.53	2.04	.06	1.27	.430
17	3.99	88.19	2.48	.45	0	10.85	3.06	1.76	.09	2.00	.495
18	6.49	73.13	2.42	.33	.79	16.84	4.14	2.36	.18	.40	.560
19	6.13	80.94	2.81	.33	0	13.06	3.95	1.76	.06	1.49	.415
Average	9.18	78.07	2.80	.32		13.97	3.48	1.92	.17	1.75	.487
Standard error	±.91	±1.67	±.36	±.025		±.59	±.19	±.10	±.02	±.19	±.102

The results of the spectrographic analyses on 12 samples of shark meal are presented in table 3. Calcium, phosphorus, sodium, magnesium, silicon, iron, strontium, manganese, zinc, lead, copper, boron, and barium were found in all of the samples. Tin was found in 8 samples, nickel in 3, and chromium in 2. Seventeen of the elements either were absent or present in quantities below the sensitivity range of the method.

TABLE 3.—*Elements detected in shark-meal samples by spectrographic analyses, using the rough estimate method* [1]

(Air-dry basis)

| Element [2] | Sample No. | | | | | | | | | | | | Average |
| | 3 | 4 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 20 | 21 | 22 | |
	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Ca	3.4000	4.9550	3.0240	3.0250	1.2830	1.3870	3.1520	3.7320	2.3040	5.3140	4.3850	2.4020	3.1970
P	1.7000	1.9800	3.0240	1.2100	1.2830	1.3870	1.5760	1.8660	1.1520	2.6570	1.7540	1.2010	1.7330
Na	.3400	.5946	.1814	.2420	.2566	.2774	.4728	.3732	.3456	.1594	.3508	.3603	.3303
Mg	.3400	.3964	.6048	.2420	.2566	.4161	.3152	.5598	.1152	.5314	.3508	.2402	.3640
Si	.1700	.1982	.0605	.1210	.0385	.0832	.1576	.1120	.1152	.0797	.1052	.0721	.1094
Fe	.0170	.0198	.0302	.0121	.0128	.0139	.0158	.0187	.0115	.0266	.0175	.0120	.0173
Sr	.0170	.0198	.0302	.0121	.0128	.0139	.0158	.0187	.0115	.0053	.0175	.0120	.0156
Mn	.0005	.0012	.0302	.0363	.0008	.0077	.0009	.0037	.0069	.0008	.0011	.0036	.0078
Zn	.0017	.0059	.0302	.0121	.0128	.0028	.0016	.0112	.0115	.0027	.0018	.0072	.0085
Pb	.0010	.0198	.0030	.0007	.0008	.0008	.0009	.0011	.0007	.0016	.0011	.0007	.0027
Cu	.0010	.0012	.0091	.0007	.0004	.0008	.0009	.0011	.0007	.0016	.0011	.0007	.0016
B	.0005	.0006	.0009	.0004	.0004	.0004	.0005	.0006	.0003	.0008	.0005	.0004	.0005
Ba	.0002	.0002	.0006	.0002	.0003	.0003	.0002	.0002	.0001	.0003	.0002	.0001	.0002
Sn	0	0	.0003	.0001	.0001	.0001	.0002	.0002	.0001	0	0	.0001	------
Ni	0	0	.0003	0	0	0	0	0	.0001	0	0	.0001	------
Cr	0	0	0	0	0	0	0	0	.0001	0	0	.0001	------

[1] The values given indicate that the quantity of a given element is estimated to lie within a range of from one-half to twice the figure given.
[2] Other elements not found in any sample by this method and their lower limits of detectability expressed as percent of the ash are: As, 0.1; Li, 0.03; W and Y, 0.02; Hg, 0.01; Cd, 0.003; and V, La, Co, Zr, Ti, Mo, Be, Bi, Sb, Ge, and Au, each 0.001.

No urease activity was observed in any of the five samples of shark meal tested for the presence of this enzyme.

Some of the nitrogen fraction A was lost during storage from each of the five samples tested. The average value for this fraction on freshly processed samples was 0.394 percent; after 70 days storage it was 0.309 percent when calculated on the basis of the original moisture content. There was no loss of urea during this study. The average

TABLE 4.—*Effect of exposing shark meal to the atmosphere during 70 days of storage on nitrogen fraction A, urea, and hydrogen-ion concentration*

| Sample No. | Nitrogen fraction A | | | Urea | | | pH | | |
| | Freshly proc- essed | After ex- posure | Effect of ex- posure | Freshly proc- cessed | After ex- posure | Effect of ex- posure | Freshly proc- essed | After ex- posure | Effect of ex- posure |
	Percent	Percent		Percent	Percent		Percent	Percent	
8	0.525	0.251	−0.074	3.35	3.36	+0.01	6.05	5.95	−0.10
9	.465	.398	−.067	1.25	1.27	+.02	5.86	5.75	−.11
10	.405	.243	−.162	1.85	1.86	+.01	5.80	5.70	−.10
11	.365	.309	−.056	2.51	2.50	−.01	5.94	5.82	−.12
12	.410	.343	−.067	.94	.95	+.01	5.92	5.80	−.12
Average	.394	.309	−.085	1.98	1.99	+.01	5.91	5.80	−.11

pH of 5.91 for the freshly processed samples decreased to 5.80 during the exposure period.   The effect of exposure of shark meal to the atmosphere for 70 days on nitrogen fraction A, urea, and pH is shown in table 4.

DISCUSSION

The total for the percentages of water, crude protein, crude fiber, ether extract, and ash exceeded 100 in many instances, probably because of the inclusion of nitrogen from nonprotein sources in the calculation of crude protein.

The proportion of skeletal material to flesh in the samples was responsible largely for the variation in mineral content and influenced inversely the percentage of crude protein.   In the processing of shark meal (*14*) there was little mixing of the material to produce a uniform product.   Shark meal is hygroscopic and its moisture content is increased by exposure to a humid atmosphere.   Samples 3 through 7, inclusive, were received during very humid weather.   The moistureproof bags in which they were shipped were damaged badly during transit and the material had absorbed considerable moisture, as indicated by the analyses (table 2).

The elements determined by the spectrographic method represent those present in the shark-meal samples after they were prepared for analysis.   Contamination with some elements possibly could have resulted from contact with the equipment used in the commercial processing of shark meal and in preparation of the samples.   The rough-estimate method of determination indicated a range within which the quantity of an element was estimated to lie.   The range was from one-half to twice the figure given.   This accounted for much of the variation reported in the composition of the samples.

Urease activity of shark-meal origin was not observed, nor was it expected since the product had been subjected to high temperatures during processing (*14*).   However, if a large bacterial population were present and conditions were favorable for their growth, urease activity might occur since it has been found (*19*) that many species of bacteria produce this enzyme.   Waksman and Davison (*20*) reported that some fungi also possess urease activity.

SUMMARY

The averages of the analyses of 19 samples of shark meal were: Water 9.18, crude protein 78.07, ether extract 2.80, crude fiber 0.32, ash 13.97, calcium 3.48, phosphorus 1.92, and magnesium 0.17 percent.   Seventeen of the shark-meal samples averaged 1.75 percent urea and 0.49 percent nitrogen fraction A.

Spectrographic analyses for 33 elements on 12 shark-meal samples showed calcium, phosphorus, sodium, magnesium, silicon, iron, strontium, manganese, zinc, lead, copper, boron, and barium to be present in all samples.   Tin was detected in 8 of the samples, nickel in 3, and chromium in 2.

In the 5 samples of shark meal tested for urease activity, none was observed.

The exposure of 5 samples of shark meal to the atmosphere for 70

days did not effect the urea content but resulted in a small loss of nitrogen fraction A and a slight increase in the hydrogen-ion concentration.

LITERATURE CITED

(1) ALMQUIST, H. J.
      1941. CHEMICAL ESTIMATION OF QUALITY IN ANIMAL PROTEIN CONCEN-
            TRATES. Jour. Nutr. 21: 347–350, illus.
(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
      1935. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 4, 710
            pp., illus. Washington, D. C.
(3) ——
      1941. CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS
            MADE AT THE FIFTY-SIXTH ANNUAL MEETING . . . Assoc. Off.
            Agr. Chem. Jour. 24: 66–101, illus.
(4) CLARK, W. M.
      1928. THE DETERMINATION OF HYDROGEN IONS . . . Ed. 3, 717 pp.,
            illus. Baltimore.
(5) EVANS, R. J., CARVER, J. S., and HAMM, W. S.
      1944. THE INFLUENCE OF STORAGE ON THE SUPPLEMENTARY PROTEIN
            NUTRITIVE VALUE OF CERTAIN FISH MEALS. Poultry Sci. 23:
            491- 494.
(6) FISKE, C. H., and SUBBAROW, Y.
      1925. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. Jour. Biol.
            Chem. 66: 375–400.
(7) GRAU, C. R., and ALMQUIST, H. J.
      1945. THE METHIONINE CONTENT OF FEEDSTUFF PROTEINS. Arch. Bio-
            chem. 6: 287- 294.
(8) GRIEM, W. B.
      1942. REPORT ON AMMONIACAL AND UREA NITROGEN. Assoc. Off. Agr.
            Chem. Jour. 25: 874–877.
(9) —— and WALKER, L. S.
      1941. REPORT ON AMMONIACAL AND UREA NITROGEN (FEEDS). Assoc.
            Off. Agr. Chem. Jour. 24: 867–871.
(10) HANDY, J. O.
      1900. THE VOLUMETRIC DETERMINATION OF MAGNESIA. Amer. Chem.
            Soc. Jour. 22: 31–39.
(11) HAWK, P. B., and BERGEIM, O.
      [1937.] PRACTICAL PHYSIOLOGICAL CHEMISTRY. Ed. 11, 968 pp., illus.
            Philadelphia.
(12) MARSHALL, S. P., BECKER, R. B., and DAVIS, G. K.
      1946. SHARK MEAL AS A PROTEIN SUPPLEMENT IN DAIRY CALF RATIONS.
            Jour. Dairy Sci. 29: 801–808, illus.
(13) —— and DAVIS, G. K.
      1946. SHARK MEAL AS A PROTEIN SUPPLEMENT IN CHICK RATIONS.
            Poultry Sci. 25: 381–386, illus.
(14) —— and DAVIS, G. K.
      1946. THE VALUE OF SHARK MEAL IN SWINE RATIONS. Jour. Anim. Sci.
            5: [211]–218, illus.
(15) MORRISON, F. B.
      1936. FEEDS AND FEEDING . . . Ed. 20, 1050 pp., illus. Ithaca, N. Y.
(16) RHIAN, M., CARVER, J. S., HARRISON, R. W., and HAMM, W. S.
      1942. THE VALUE OF DOGFISH MEAL AS A PROTEIN SUPPLEMENT. Wash.
            Agr. Expt. Sta. Bul. 416, 24 pp.
(17) ROBERTS, J. A., and GADDUM, L. W.
      1937. COMPOSITION OF CITRUS FRUIT JUICES. Indus. and Engin. Chem.
            29: 574–575.
(18) SNEDECOR, G. W.
      1937. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE
            AND BIOLOGY. 341 pp., illus. Ames, Iowa.
(19) SOCIETY OF AMERICAN BACTERIOLOGISTS.
      1939. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY . . . Ed. 5,
            1032 pp. Baltimore.
(20) WAKSMAN, S. A., and DAVISON, W. C.
      1926. ENZYMES; PROPERTIES, DISTRIBUTION, METHODS AND APPLICA-
            TIONS. 364 pp., illus. Baltimore.

TRANSFER OF THE MOSAIC-RESISTANCE FACTOR BETWEEN H CHROMOSOMES OF NICOTIANA GLUTINOSA AND N. TABACUM[1]

By D. U. Gerstel [2]

Formerly *associate in genetics, California Agricultural Experiment Station*

INTRODUCTION

Mosaic-resistant Holmes Samsoun tobacco was established by substitution of a *Nicotiana glutinosa* chromosome carrying resistance for the H chromosome of Samsoun tobacco. The advantages of this accomplishment from the point of view of the tobacco breeder were, however, marred by inclusion of *glutinosa* factors detrimental to yield linked with resistance in the "alien substitution race." According to Valleau (8),[3] several years of backcrossing have failed to separate the resistance factor or factors from those that reduce yield. Evidently failure of pairing of the *N. tabacum* H_t chromosome with its *glutinosa* H_g equivalent in the hybrid prevents exchange of segments and the consequent recombination upon which hopes of improvement rest (for details and references see (3)). However, recent evidence, as set forth in the present paper, indicates that an occasional exchange of segments may occur between these two chromosomes.

EXPERIMENTAL DATA

In 1944 seeds of mosaic-resistant burley tobacco which had been derived from Holmes Samsoun by backcrossing to burley and selfing were received from the Plant Industry Station, Beltsville, Md. Three crosses between homozygous resistant plants and either nonresistant burley or the nonresistant variety Purpurea used as a standard at Berkeley were grown from 1944 to 1946. The following cytological observations were made in pollen mother cells of the progeny.

Meiotic behavior was determined in 280 metaphase plates of 3 different crosses, nonresistant×homozygous resistant (table 1). Of these 212 exhibited 24 pairs, while the remainder had an unassociated pair of univalents (or 2 in 2 cases). While it is not possible to identify with certainty a univalent H chromosome because of its similarity

to other chromosomes of the complement (*2*), the univalents in the $23_{II}+2_I$ column of table 1 did not differ perceptibly from the medium large type represented by H. Presumably, then, H is involved in the nonconjunction in all or most cases.

Plant No. 45264p21, which had exhibited no nonconjunction among the small number of PMC's studied, was chosen as the male parent for a cross with a haplo-H plant of the Purpurea variety (table 2). The progeny (46458) segregated independently for haplo and diplo types (37:4 is in fair agreement with the female transmission rate of haplo-H as given by Clausen and Cameron (*2*)) and for mosaic resistance (14 resistant : 25 susceptible fits only poorly the expected 1:1 ratio, $P < .20$). Metaphase plates of selected resistant plants gave the following results (table 2) : All 19 PMC's from 4 different haplo-H plants showed $23_{II}+1_I$. Here no nonconjunction was observed among the 23 pairs. But among 18 metaphase figures from 1 resistant diplo-H plant, 46458p28, there were 4 with nonconjunction of H-type chromosomes. Thus the low degree of nonconjunction between the resistance-bearing chromosome and its partner, which was demonstrated in plant 45264p21 and in its siblings, was carried over into the next generation.

TABLE 1.—*Cytology of F_1 nonresistant varieties $\times$ resistant burley*

Cross	Population and plant No.	24_{II}	$23_{II}+2_I$	$22_{II}+4_I$
Burley BR 645 A $\times$ resistant burley No. 1.	44180p1	8	1	0
	44180p5	6	1	0
	44180p7	9	0	0
	44180p8	9	11	0
	44180p10	9	0	0
	45264p3	8	2	0
	45264p4	7	0	0
	45264p6	7	0	0
	45264p21	10	0	0
	46454p16	6	2	0
Burley BR 645 A $\times$ resistant burley No. 2.	44181p7	4	8	0
	45265p3	0	5	1
	45265p3a	11	0	0
	45265p4	10	0	0
	45265p7	9	1	0
Purpurea $\times$ resistant burley No. 1	45266p2	8	2	0
	45266p15	6	4	0
	46455p2	14	4	0
	46455p5	29	1	0
	46455p7	9	6	0
	46455p13	9	3	0
	46455p26	5	4	1
	46455p28	19	11	0
Total		212	66	2

TABLE 2.—*Cytology of Purpurea haplo-H $\times$ 45246p21*

Population and plant No.	Number of chromosomes	$23_{II}+1_I$	$23_{II}+2_I$	$22_{II}+4_I$	24_{II}
46458p2a	47	5			
46458p20	47	6			
46458p29	47	2			
46458p36	47	6			
46458p28	48		3	1	[1] 14

[1] Including 1 PMC with 1 very large unconjugated pair resembling the F chromosomes, which occasionally fail to pair in normal material.

Since the H_g chromosome was originally unable to pair with H_t the newly acquired pairing ability must rest upon some modification, presumably a translocation [4] between the H_g and H_t chromosomes, as indicated by the following evidence: (1) The nonconjoined chromosomes in tables 1 and 2 (with the exceptions noted) are of the general type resembling H; this, of course, is not very weighty evidence. (2) The disomic plant No. 46458p28 (table 2), which contained an unmodified H_t chromosome from the Purpurea parent and a modified chromosome from its male parent, showed some nonconjunction. On the other hand, its monosomic siblings with no H_t chromosome derived from the male parent exhibited no nonconjunction of pairs. Thus the H pair was responsible for the nonconjunction observed in the disomic plants.

To prove conclusively that resistance is still carried in an H chromosome and has not been translocated to some other chromosome, a population was grown from a pollination of normal Purpurea with plant 46458p36, one of the resistant haplo-H plants in table 2. If this monosomic plant carried its resistance in some other chromosome, rather than in the H chromosome, its expected progeny should conform to a 1 : 1 ratio. If, however, resistance was located in the H chromosome, then the great majority of the pollen offspring would be resistant. In all the monosomics of *tabacum* tested (6) pollen grains with 23 chromosomes showed little ability to compete with those having a full complement. The exact value for the transmission of the monosomic condition has not been determined as yet for the haplo-H type, and upon it depends the frequency of nonresistant plants in the progeny if resistance should be located in H. Also the resistance factor may occasionally become deleted by fragmentation from the H monosome, and again susceptible plants would result. The following values were actually obtained in the experiment: Out of a total of 93 plants (obtained from 130 seeds) 84 proved resistant after inoculation and only 9 were nonresistant. This does not fit a 1 : 1 hypothesis ($P<.01$), even if due allowance be made for seeds which failed to germinate, and the assumption is justified that a modified H chromosome is the carrier of resistance.

DISCUSSION

Modification of chromosomes has been observed frequently before in plants with univalents. Clausen (1), Stino (7), and Tobgy [5] have described fragmentation and translocation in the progeny of monosomic tobacco.

Several mechanisms have been held in the past to account for translocation. The present data have little bearing on the question whether translocation is a process consisting of two separate steps, namely, breakage followed by fusion of exchanged ends (5), or whether it is a result of crossing over between nonhomologous associates (4).

[4] "Translocation" is used here to designate an exchange between the two nonpairing chromosomes; the term is usually defined more specifically as an attachment of a sector from one chromosome to a nonhomologue.

[5] Tobgy, A. H. A cytogenetic study of some chromosomal alterations in a monosomic type of Nicotiana tabacum, 96 pp. 1946. [Unpublished doctoral thesis. Copy on file in library, University of California, Berkeley.]

SEGREGATION IN RUSSETED SPORTS OF THE GRIMES APPLE [1]

By V. R. GARDNER, director, Michigan Agricultural Experiment Station, W. TOEN-
JES, superintendent, Graham Horticultural Experiment Station, and M. GIEFEL,
research assistant, Michigan Agricultural Experiment Station

INTRODUCTION

Clonal varieties of plants, being vegetatively propagated, are gen-
erally considered to be characterized by a high degree of uniformity.
Indeed, it is sometimes said that the different individuals comprising
them are practically identical with each other and with their parents
in form and behavior. Uniformity, however, is a relative term.
Some clonal varieties, e. g., the Concord grape, are comparatively
uniform; others, e. g., the Boston fern, are much more variable. How
uniform, or how variable, are the individuals of a clonal variety is
often a matter of considerable importance to the nurseryman and
likewise to the producer of fruits and ornamentals, for they are
obliged to meet certain standards of uniformity or have their products
graded down and suffer consequent losses.

It has been known for centuries that rather striking variations now
and then appear in vegetatively propagated varieties. These have
been called bud sports or mutations and some of them in turn have
been propagated as new varieties. With these bud sports, as with
their parents, it has been generally assumed that they are stable and
uniform in character. Some studies during recent years, both of
well known clonal varieties [2][3] and of certain bud sports [4][5][6][7], have
shown that their variability is greater than has been generally thought.
This is especially true of those varieties that are commonly classified

[2] GARDNER, V. R. STUDIES IN THE NATURE OF THE CLONAL VARIETY. III.
PERMANENCE OF STRAIN AND OTHER DIFFERENCES IN TREE SIZE IN THE MONT-
MORENCY CHERRY. Mich. Agr. Expt. Sta. Tech. Bul. 186, 20 pp., illus, 1943.
[3] GARDNER, V. R., and BATEN, W. D. STUDIES IN THE NATURE OF THE CLONAL
VARIETY. II. SELECTION WITHIN A PERICLINAL CHIMERA. Mich. Agr. Expt. Sta.
Tech. Bul. 179, 48 pp., illus. 1942.
[4] GARDNER, V. R. STUDIES IN THE NATURE OF THE POMOLOGICAL VARIETY.
I. A HETERO-CHIMERIC APPLE SPORT AND ITS VEGETATIVE PROGENY. Mich.
Agr. Expt. Sta. Tech. Bul. 161., 14 pp., illus. 1938.
[5] GARDNER, V. R. A STUDY OF THE SWEET-AND-SOUR APPLE CHIMERA AND
ITS CLONAL SIGNIFICANCE. Jour. Agr. Res. 68: 383–394, illus. 1944.
[6] GARDNER, V. R., CRIST, J. W., and GIBSON, R. E. SOMATIC SEGREGATION IN A
SECTORIAL CHIMERA OF THE BARTLETT PEAR. Jour. Agr. Res. 46:1047–1057.
1933.
[7] NEWCOMER, E. H. STUDIES IN THE NATURE OF THE CLONAL VARIETY. IV.
CYTOLOGICAL STUDIES OF BUD SPORTS OF MCINTOSH, STARK AND BALDWIN APPLES.
Mich. Agr. Expt. Sta. Tech. Bul. 187. 23 pp., illus. 1943.

as chimeras. A study of a large number of bud sports of clonal varieties of apple, pear, cherry, and pelargonium at the Michigan station during the last 25 years has served to emphasize the variable, rather than the uniform, characteristics of many of them. The behavior of certain bud sports propagated for the first time from the parent limb has been especially useful in throwing light on this question.

DESCRIPTION OF MATERIALS

Among a considerable number of russeted apple sports of normally smooth-skinned varieties in the collection of the Michigan Experiment Station are two of the Grimes. One of these, designated as No. 483, was found as a limb variation in a tree in Oceana County, Mich. This limb was brought to the attention of one of the writers in the twenties. It was observed for several successive years when its fruit was approaching the harvesting stage. Its fruits were thinly covered with russet, though not as distinctly russeted as most so-called russet varieties (e. g., Golden Russet, Roxbury, Pumpkin Russet, etc.). The other, designated as No. 581, was a limb sport with similar characteristics on a tree near Montrose, Mich.

Scions were taken from both of these limb sports and a number of nursery trees were grown from them. Each nursery tree grew from a single scion bud. Five trees of No. 483 and three of No. 581 were set in the orchard in 1932 and 1934 respectively. Some of these trees bore a few fruits in 1936 and 1937, but it was not until 1938 that the crops borne by any of them were large enough to warrant making careful records of the amount and distribution of their russeting

PRESENTATION OF DATA

When the trees of these russeted Grimes sports bore their first fruits it was noted that there were rather marked differences from tree to tree in the amount of russeting. Some trees bore relatively smooth, others relatively russeted fruits. On some of the trees the fruits throughout the tree were comparatively uniform in the amount of russeting; on others there was considerable variation from branch to branch. (See fig 1.) In one instance this difference between branches was so marked that it was decided to harvest the fruits from them and record the data separately. This particular tree had six main branches, labeled A. B, C, D, E, and F; one of these (D) produced a strong lateral that was labeled D_1, the fruits of which were substantially different from those of its parent branch.

The crops borne by the different trees of these two russeted Grimes sports varied in amount from year to year. In certain years (1938, 1942, 1943, 1944, 1945, and 1946 in the case of No. 483 and 1940, 1942, 1943, and 1946 in the case of No. 581) either the entire crop or random samples of 1 or more bushels were harvested from each tree, and each fruit was examined and its percentage of russeted surface estimated and recorded. In other years, because of the small size of the crops or the limitations of time, such detailed records were not obtained. However, in those instances observations were made and notes taken which serve to substantiate the more detailed records made during the other seasons.

FIGURE 1 —Typical fruits from a single tree of Grimes sport No. 483.   These
fruits show gradations in russeting ranging from a completely smooth (at the
upper right) to a completely russeted (at the lower right) surface.

In tables 1 and 2 detailed data are presented for the 1946 crop season
and summarized data for earlier years, showing the percentages of the
fruit surfaces covered by russet.   These data are presented for each
tree of both sports and for each of the several branches of one of the
trees of No. 483.

Attention is called first to the marked differences from year to year
in the amount of russeting.   The seasons of 1942, 1944, and 1945 were
characterized by heavy russeting; 1938, 1943, and 1946 were charac-
terized by light russeting; 1940 was intermediate in this respect.

After making due allowance for the influence of season on russeting,
it is obvious that different trees of the same selection (i. e., No. 483 or
No. 581) not only showed marked differences in the amount of rus-
seting in any one season but these differences continued to manifest
themselves year after year.   Thus in the case of No. 581, one tree
(10S) showed almost complete reversion to the normal smooth skin, a
second tree (10R) regularly bore rather heavily russeted fruits, while
the third tree (9R) regularly bore rather lightly russeted fruits.   The
differences between trees 4S, 5S, 3R, and 4R or No. 483 were not so
distinct; but those between the different limbs of tree 3S were equally
distinct and appeared to be equally fixed.   In all of the trees, except
10S (No. 581) and in all of the branches of tree 3S (No. 483) except A,
both of which had more or less completely reverted to the parent form,
there was rather wide variability in the amount of russeting.

TABLE 1.—Frequency distribution table showing the percentage of russeted surface on fruit of different trees and branches of Grimes sport No. 483 in 1946; mean percentages are given for 1938, 1942, 1943, 1944, and 1945

Percent of surface russeted in 1946	Tree No.										
	4S	5S	13R	14R	3S, branch						
					A	B	C	D	D₁	E	F
0	27	20	42	129	422	9	4	19	150	6	42
10	25	12	11	16	64	7	26	57	26	9	46
20	91	50	7	23	2	41	56	126	12	64	166
30	170	114	36	75		109	115	145	7	162	236
40	58	45	32	46		69	71	85	7	113	120
50	30	22	50	36		45	45	50	7	67	80
60	24	25	43	22		38	25	26	4	46	43
70	10	3	29	5		17	6	13		17	18
80	8	2	13	2		8	3	9	2	15	10
90	1	4	5			5	1	2	2	4	3
100	1		1								
Total	445	297	278	354	488	348	355	532	217	503	764
Mean	31.4	32.6	42 3	20 7	1 4	39.4	35 0	31 2	9 0	39 0	29 7
Means for seasons of:											
1938	36 5				6 4	60 0	55 0	43 6	15.0	37. 0	46 7
1942	78 3	88. 0	50 7	59 9	10 0	84 5	50. 0	59. 4		80 4	82. 0
1943	31 3	28. 8	44 5	44 8	7 3	41 0	21. 4	36. 0	16 4	46. 0	30 9
1944	67 7				18. 8	73 4	68 5	60. 7		72 5	63 1
1945	73.7	70. 2	36 4	65 2	. 8		84 5	74 2		81. 2	69 6

¹ The apparent bimodal picture presented by the 1946 figures for these 2 trees is due to the fact that both of them, like tree 3S, have certain limbs whose fruits show almost complete reversions to the smooth condition of the normal Grimes.

TABLE 2.—Frequency distribution table showing the percentage of russeted surface on fruit of different trees of Grimes sport No. 581 in 1946; mean percentages are given for 1940, 1942, 1943, and 1945

Percent of surface russeted in 1946	Tree No.			Percent of surface russeted in 1946	Tree No.		
	9R	10R	10S		9R	10R	10S
0			198	100			
10	35	8	24				
20	91	21	3	Total	310	259	225
30	98	45		Mean	29 8	49 2	1. 3
40	39	30		Means for seasons of:			
50	32	42		1940	58. 1	70. 4	3. 7
60	11	46		1942	94. 1	99 7	8. 9
70	3	31		1943	41. 1	49. 6	5. 5
80	1	26		1945	86. 5	91. 3	. 6
90		10					

DISCUSSION

The data presented suggest that the several trees, or limbs, were perhaps as a whole no more fixed in their character than were the original limb sports from which they were propagated. Obviously the original limb mutations were chimeras (i. e., tissue mixtures); so were some, if not all, of the trees propagated from them. Buds taken from any one of them might yield trees essentially the same as the parent or they might show the diversity that characterizes their own vegetative generation. In their individual tree uniformity and fixity and also in their variability from tree to tree they resemble the sectorial chimera strains of the striped Bartlett pear described by Gardner,

Crist, and Gibson [8] and the heterochimeric Graham apple described by Gardner [9]. Yet on the basis of casual observation these trees would readily be classed simply as a russet variety.

Careful observations and many detailed records that have been made of bud sports of many kinds (color, size, shape, etc., many of which cannot be quantitatively measured or accurately classified as a russeted surface) in the Michigan station's collection suggest strongly that a large proportion of them are of the same basic nature as the two russeted forms here described. This in turn strengthens the practical intrepretation placed on data presented in an earlier report,[10] namely:

. . . both nurseryman and grower should realize that there are strain differences . . . and nurserymen should propagate and growers should plant those strains whose superior growing and producing qualities have been definitely established as a result of carefully conducted trials.

To this may be added the statement that intravarietal variability is greater than has been generally assumed.

SUMMARY

The characteristics of daughter trees propagated from russeted limb sports of two Grimes apple trees are described.

Some of these daughter trees showed reversions to the normal smooth parental type.

Others showed segregation in respect to amount or percentage of russeting. These segregates exhibited a marked degree of permanence or fixity of type.

Certain trees produced individual limbs showing similar reversion and segregation.

Some practical implications of these characteristics of sporting forms are pointed out.

---

[8] See footnote 6, p. 225.
[9] See footnote 4, p. 225.
[10] See footnote 2, p. 225.

VARIABILITY AND SEGREGATION IN THE GOLDEN RUSSET APPLE [1]

By V. R. Gardner, *director, Michigan Agricultural Experiment Station,* W. Toenjes, *superintendent, Graham Horticultural Experiment Station,* and M. Giefel and J. C. Kremer, *research assistants, Michigan Agricultural Experiment Station*

INTRODUCTION

Acceptance of the concept that the clonal variety is highly uniform and fixed, at least as compard with the seminally propagated variety, has been general, if not universal. Different individuals of the same clonal variety are often referred to simply as different parts of the same individual and are regarded as practically identical.

Studies of a large number of bud sports of certain deciduous fruits that have been under way at the Michigan Agricultural Experiment Station for a quarter of a century have served to draw attention to their relatively great diversity rather than to their uniformity. Several reports have been made (*2, 4, 6, 7*) [2] describing these intrastrain diversities and pointing out their possible significance. At the same time that the bud sports have been under study, corresponding observations have been made of certain of their parent forms. Most of these parent forms are standard well-known *varieties (3, 5, 8)*. These varieties are obviously different from their bud sports; otherwise the sports would not be recognized as such. On the other hand, when the parent forms have been compared with their bud sports in such basic characters as uniformity or variabilty, some of them appear to be very much like their progeny. Data are presented here on studies made on one of these varieties, the Golden Russet apple.

DESCRIPTION OF MATERIAL AND PROCEDURE

The time and place of origin of the Golden Russet apple are not known. It was brought to this country from England in colonial days, and has been widely propagated by many nurseries and extensively planted, particularly in the Northeastern States.

In describing its fruit, Beach (*1, p. 144*) states:

Skin . . . sometimes only partly covered with patches and flecks of russet but more often almost entirely covered with green or yellowish russet, in highly colored specimens becoming golden russet with bronze cheek. *Dots* grayish or

russet, rather inconspicuous on the smooth skin but on the russet skin often clear pale gray and conspicuously scattered over the base.

Descriptions of other pomologists agree closely with that of Beach. All classify it as a russet variety, with no suggestion of more variability in the amount or percentage of the fruits' surface being russeted than is true of other varieties of its class. There is, however, in the description of Beach and in those of other writers recognition of some variability in this respect.

The trees of this variety at the experiment station, which furnished the fruits for some of the earlier observations and later the scions for producing another vegetative generation of trees, were 60 to 70 years
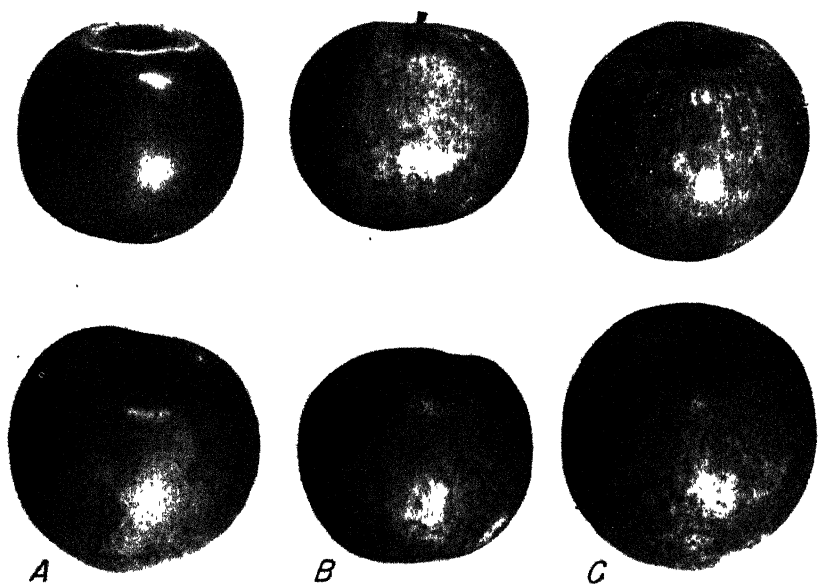
FIGURE 1.—Photographs showing differences in amount of russeting of fruits of the Golden Russet apple. *A*, fruits practically smooth; *B*, about one-fourth russeted; *C*, three-fourths russeted. All fruits are from the same tree

old at the beginning of the study. They appeared to be normal in every respect, similar to thousands of other trees of the same variety in other orchards of the State. Russeting of their fruits could be accurately described by the quotation from Beach in the preceding paragraph. These differences in surface are illustrated in figure 1. It was noted by one of the authors, however, that a large limb of one of these old trees bore fruits that were completely and very heavily russeted, a rather striking bud sport. (See fig. 2.) The occurrence of this limb sport led to close examination of the degree or amount of russeting of fruits borne by other parts of the tree and by other trees of the variety in the same orchard and likewise in a number of other orchards. These observations revealed a wide variation in the amount of russeting on the different fruits, from almost completely covered to almost completely smooth. For the most part these fruits with different amounts of russeting were scattered at random throughout the

trees. Relatively smooth specimens might be borne on spurs next to spurs bearing heavily russeted or semirusseted specimens; or a single spur might bear fruits showing both extremes. Here and there, however, branches of varying size were noted whose fruits seemed to be predominantly rather lightly russeted, or predominantly rather heavily russeted; still others bore fruits showing the entire range in degree of russeting.

It was decided that the observations were interesting enough to warrant a somewhat more extended study. Two large trees in the experiment station orchard at Grand Rapids were selected for it. Five thousand numbered metal tags were fastened to as many individual spurs on these two trees in the fall of 1933 just before harvest.
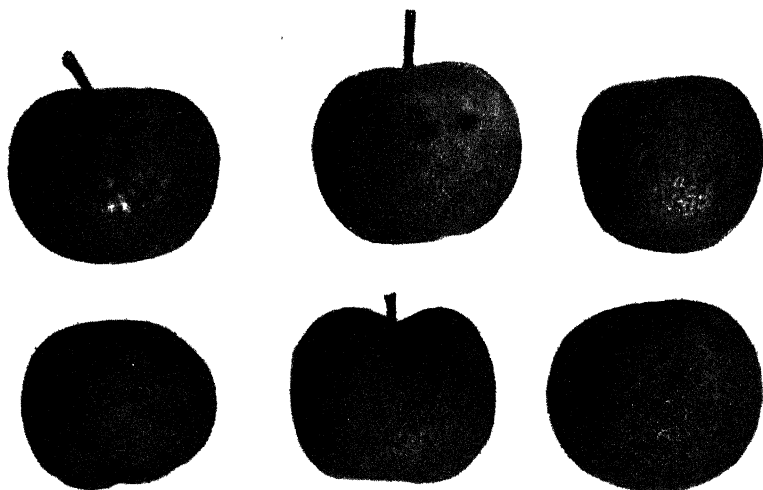
FIGURE 2.—Typical fruits on a limb sport of the Golden Russet apple at the Graham Horticultural Experiment Station, Grand Rapids, Mich. Note the thick, deep character of the russet and the tendency of the skin to crack and scale off

Each apple harvested from a numbered spur was then examined and classified roughly as to amount of russeting by placing it into one of five groups: Completely russeted or practically so, three-quarters russeted, one-half russeted, one-fourth russeted, smooth or almost smooth. Suitable records were made of the character of the fruits borne by each numbered spur. Fruits borne by these same numbered spurs each year for the following 6 years were similarly classified as to amount of russeting and records kept so that one year's performance of a certain spur could be compared with its earlier or later performance and likewise the mass performance of a whole group of spurs on a limb could be compared year after year.

No one spur bore fruits each and every year throughout the 7-year period. Indeed, there were many for which only a single year's performance was available, for there were many breakages, losses of labels, etc. However, there were also many spurs that bore fruit several times during the period in question, enough to furnish some evidence

of the degree of fixity of this characteristic of the fruit in the spur or branch on which it was borne.

Scions were cut from certain limbs selected as producing fruits that fell mainly in one or another of the above classes or that represented certain intermediate conditions. Nursery trees were grown from these scions in the usual manner, each tree developing from a single bud of its scion. Seventy-two trees were set in the orchard in the spring of 1935. The same spring that a part of the scions of these numbered selections were used for bench grafting (1933), others were set as top grafts in already established orchard trees.

Some of these top grafts bore a fairly large number of fruits in 1939 and the orchard trees began producing a year or two later. When either a top graft or an orchard tree bore a considerable number of fruits either fairly comprehensive notes were taken on their russeting or they were harvested and each specimen examined and classified for russeting.

PRESENTATION OF DATA

The data that have been collected furnish at least partial answers to three questions: (1) The influence of seasonal conditions on the amount of fruit russeting, (2) the branch to branch variation within the tree in respect to amount of russeting and the permanence or fixity of those differences, and (3) the possibility of segregating and propagating intravariety strains that differ from each other in amount or degree of russeting.

INFULENCE OF SEASONAL CONDITIONS ON AMOUNT OF FRUIT RUSSETING

Table 1 summarizes the data for the seasons 1933–39, inclusive, on the amount of russeting on the fruits of each of the two parent Golden Russet trees, in the experiment station orchard at Grand Rapids, Mich. In 1933 the fruits russeted rather heavily; in 1934 and 1936 they were almost completely smooth skinned, slightly russeted in 1935 and 1937, and moderately russeted in 1938 and 1939.

TABLE 1.—*Mass performance of 2 Golden Russet trees in respect to russeting of fruit for the seasons 1933–39, Grand Rapids, Mich.*[1]

Year	Total number of fruits examined for russeting		Mean percent of surface russeted	
	Tree No. 1	Tree No. 2	Tree No. 1	Tree No. 2
1933	2,060	1,577	43.1	37.8
1934	2,109	1,150	16.0	.5
1935	3,583	4,320	21.5	6.7
1936	1,401	1,721	12.3	.8
1937	2,991	3,750	19.1	6.5
1938	554	1,961	11.6	14.1
1939	[2]60	16,091	[2]41.7	18.6

[1] 1936 was a light crop year for both trees. Extremely few records were obtained in 1939 for tree No. 1 because of lack of time.
[2] Sample too small to fairly represent the tree.

TREE, BRANCH, AND SPUR DIFFERENCES IN RUSSETING

There are likewise tree and branch differences that are reasonably consistent from season to season. Thus the furits of tree No. 1 (Table 1) were on the whole more heavily russeted than those of tree

TABLE 2.—Mass performance of selected branches of 2 large Golden Russet trees in respect to russeting of fruit for the seasons 1933-39, Grand Rapids, Mich.

Year	Total number of fruits examined for russeting							Mean percent of surface russeted						
	Tree No. 1			Tree No. 2				Tree No. 1			Tree No. 2			
	Br. 2AA	Br. 5B	Br. 6C	Br. 1B	Br. 2D1	Br. 4A1	Br. 6A	Br. 2AA	Br. 5B	Br. 6C	Br. 1B	Br. 2D1	Br. 4A1	Br. 6A
1933	195	[1]46	137	[1]59	96	[1]20	92	26.5	[1]99.4	37.0	19.5	11.8	[1]62.5	47.3
1934	267	139	[1]27	188	160	[1]13	126	14 4	98.7	[1]25.0	0	.2	[1]0	0
1935	231	115	170	332	183	138	[1]4	14.1	85.2	12.8	4 1	6 6	11.2	[1]7.6
1936	90	[1]6	[1]2	[1]44	[1]4	[1]4	91	13.3	[1]83.3	[1]0	[1]3.4	[1]12.5	[1]6.2	5.5
1937	214	263	124	294	168	126	218	22.9	81.2	6 7	6.3	5.7	6.8	4.2
1938	[1]38	------	[1]2	83	90	[1]49	79	[1]21 1	------	[1]12.5	17.8	20 6	[1]24.5	1 3
1939	[1]21	------	[1]2	4,415	694	249	1,372	47.6	------	50.0	6.8	33.0	47.0	11.1

[1] Samples too small to fairly represent the branches.

No. 2. The fruits borne on branches 5B of tree No. 1 and 4A1 of tree No. 2 (table 2) similarly were more heavily russeted than the general tree averages, while branches 6C of tree No. 1 and 1B of tree No. 2 bore fruits less russeted than the general tree averages. In certain seasons these differences were relatively slight. Apparent inconsistencies are probably to be explained by the relatively small numbers of fruits borne by certain limbs in certain seasons—numbers too small to be truly representative of the branch in question.

None of the 5,000 spurs for which records were obtained bore fruits more than 5 times in the 7-year period covered by this study; only a few fruited 4 times; many fruited 3 times. Some of the spurs that bore 3 or 4 times were consistent in producing only smooth or only half-russeted fruits each time; more of them varied from year to year. Thus spur No. 268 produced a smooth-skinned fruit in 1933, 1 that was a quarter russeted in 1935, a smooth one in 1937, 2 smooth ones in 1938, and 1 that was a quarter russeted in 1939. On the whole, individual spur performance in one season gave little indication of what to expect in succeeding seasons. Thus the 346 spurs of tree No. 2 that produced smooth fruits in 1933 produced 69 smooth, 8 quarter-russeted, 2 half-russeted, and 1 fully-russeted fruits in 1935, while the 350 spurs that produced three-fourths russeted fruits in 1933 produced 77 smooth, 20 quarter russeted, 1 three-quarters russeted, and 1 fully russeted in 1935. Their performance in 1936–39 showed similar distributions.

PROPAGATION TRIALS WITH RUSSETING VARIATIONS

When tree branches that appeared to be somehwat different from others in the amount of russeting of their fruits, but whose fruits nevertheless showed considerable diversity in this respect, were put to the propagation test, their daughter trees taken together produced fruits that would classify them simply as Golden Russet. Their fruits were neither more nor less heavily russeted than those of trees of this same variety in other orchards. Closer examination, however, revealed certain rather consistent individual tree and group differences. A large number of detailed records were obtained. Representative data are presented in tables 3 to 5.

TABLE 3.—*Frequency distribution table showing percentage of surface russeted on fruits of 3 daughter trees of Golden Russet selection No. 485*

Year and tree No.	Number of fruits showing following percent of russeting																					Total	Mean percent of surface russeted
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
1939																							
Tree S6	6	2	4	2	3	4	2		7	1	2		3	1		7	5	4	8	10	7	78	59.0
Tree S7																							
1940																							
Tree S6																			1	1	20	22	99.3
Tree S7																			2	9	128	141	99.2
1941																							
Tree S6																					6	6	100.0
Tree S7									1		2		2		2		2		6		30	45	91.1
1942																							
Tree S6											1										63	64	99.2
Tree S7															1		5		3	10	404	423	99.9
1944																							
Tree S6													3		6		14		49		175	247	96.1
Tree S7																			36		188	224	98.4
1945																							
Tree A1								1							1		2		5		233	242	99.2
Tree B1																					18	18	100.0
1946																							
Tree A1													1		1		4		50		238	294	97.8
Tree B1															1		20		69		203	293	96.2
Tree C1													2		9		23		46		228	308	95.9

TABLE 4.—*Frequency distribution table showing percentage of surface russeted on fruits of 3 daughter trees of Golden Russet selection No. 544*

Year and tree No.	Number of fruits showing following percent of russeting																					Total	Mean percent of surface russeted
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
1941																							
Tree 17A																							
Tree 17B																							
Tree 17C	5	17	21	16	9	7	8	5	5	2	3	1	6	1	3	4	3	1				117	26.3
1942																							
Tree 17A		5			18	6	4		6	6	14	1	1	6	3	7	1	1	3	6	6	91	49.5
Tree 17B						1	1		1	1	1		2			2				2		12	56.8
Tree 17C							4		2	1		1	1			3	2	2	9	9	13	46	83.4
1944																							
Tree 17A							3		3		3		6		5		6		9		14	51	74.5
Tree 17B																							
Tree 17C							1		3		11	1	12		10		9		8		2	57	66.3
1945																							
Tree 17A	13		80		38		39		17		8		13		7		2				9	237	24.6
Tree 17B						129					34					12						184	36.5
Tree 17C	17		63		50		39		21		13		9		8		2					222	25.0

TABLE 5.—*Frequency distribution table showing percentage of surface russeted on fruits of 3 daughter trees of Golden Russet selection No. 545B*

Year and tree No.	Number of fruits showing following percent of russeting																					Total	Mean percent of surface russeted
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100		
1941																							
Tree 2D	1	4										1										6	12.5
Tree 2E	10	14	6	2	2			1	2													37	8.5
Tree 2F																						--	--
1942																							
Tree 2D	3	18	7	11	2	5	5	8	2	5			2		1		3		1			81	26.7
Tree 2E	1	54	2	9	15	3		4	10	2			3		1			2		2		123	22.7
Tree 2F		13	15	4	4	3	11	4		2		3	2	2	2		1	2	1	1	1	63	29.7
1944																							
Tree 2D	1		6		7		8		7		7		7		6	4	4		4			57	45.4
Tree 2E			6		10		15		10		14		13		8	6	9		10		9	113	58.5
Tree 2F	1		4		15		12		12		11		14		11	3	10		6		1	97	49.5
1946																							
Tree 2D	28		71		58		34		25		20		0		10		4		3		2	204	26.2
Tree 2E	85		119		60		12		10		4		1									291	11.8
Tree 2F	17		75		51		27		14		8		3		1		1					197	18.8

The two trees propagated as selection No. 485 from the heavily russeted east limb "sport" rather consistently produced heavily russeted fruits year after year (table 3). Several top grafts of this same selection similarly produced heavily russeted fruits. However, a few fruits borne by these trees and likewise by the top grafts were not entirely covered by russet. Furthermore, hardly any were as thickly russeted as the fruits of the parent limb; their surfaces were completely and evenly covered with a medium thin to thick russet, not the thick, rough covering characterized by checking and scaling pictured in figure 2. No explanation is offered as to why in 1939 tree S–6 of this selection showed such extreme variability in amount of russeting, when it was so much more uniform in this respect in other years.

Each of the daughter trees of selection No. 544 showed wide variation in the amount of russeting of its fruit, but the means for the fruits on each tree were reasonably uniform and were about average for all trees of the Golden Russet variety. Thus they resembled the parent limb from which the scions were taken.

The fruits borne by the three daughter trees of selection No. 545B similarly showed great diversity in amount of russeting, but the percentages of their surfaces covered with russet averaged distinctly lower than those of the daughter trees of selections 485 or 544.

DISCUSSION

The interpretation to be placed upon the data that have been presented on russeting in the Golden Russet apple is that, at least in respect to russeting,[3] this variety behaves like a chimera. This concept would explain not only its great variability but also its tendency to produce segregating limbs and whole trees that exhibit a considerable degree of permanence or fixity in their deviation from the mean or average for the variety. Intravarietal russet strains, comparable to certain chimeral strains of the Graham apple (*2*), Bartlett pear (*6*), and Madame Salleron pelargonium (*5*), originate in the same way. Like them, however, they appear to be rather unstable, ever-sporting strains, whose type can be maintained only by continuous rigid selection. This emphasizes some of the statements made at the beginning of this article to the effect that the Golden Russet apple is characterized more by variability than it is by uniformity.

If the Golden Russet is a chimera, possibly many of the other russeted varieties are likewise chimeras, for observations indicate that they closely resemble it in the matter of russeting. So far as the writers are aware from a review of the literature, russeted apple varieties have not been considered chimeras. They have simply been thought of as ordinary clonal varieties and therefore a priori as characterized by a high degree of uniformity. This raises the question: What percentage of our ordinary clonal varieties are uniform in the sense that they have been so considered and what percentage are in reality chimeras and therefore highly variable?

[3] Observations, in the form of qualitative organoleptic tests, that have been made year after year lead to the belief that it is chimeric in other respects, resembling the Sweet-and-Sour variety described by Gardner (*4*). Smooth-skinned fruits are mild subacid in flavor, somewhat resembling Rhode Island Greening; russeted fruits are sweeter to the taste, spicy, and have a touch of astringency.

SUMMARY AND CONCLUSION

Examination and classification of the fruits borne by 5,000 spurs on 2 Golden Russet apple trees over a 7-year period showed that:

(1) There are large differences in the amount of russeting from year to year, to be attributed to climatic influences.

(2) Individual trees, and likewise individual branches, are characterized by great diversity in any one season in respect to russeting.

(3) In spite of this diversity within branches and within whole trees there are substantial differences between whole trees and between branches of the same tree in respect to russeting of fruit.

Examination and classification of fruits borne over a 7-year period by 72 daughter trees propagated from selected Golden Russet limbs showed:

(1) Varying degrees of diversity within individual trees, comparable to that of the parent trees from which the scion wood was taken.

(2) Individual tree differences and also in some instances differences between groups of trees of the same selection that were more or less constant from year to year.

The data obtained from a study of fruit russeting on both parent and daughter trees lead to the conclusion that the Golden Russet apple variety is a chimera. This suggests that many other ordinary varieties similarly may be chimeras.

LITERATURE CITED

(1) BEACH, S. A.
    1905. THE APPLES OF NEW YORK. Vol. 1, 409 pp., illus. Albany, N. Y.
(2) GARDNER, V. R.
    1938. STUDIES IN NATURE OF POMOLOGICAL VARIETY. I. A HETERO-
       CHIMERIC APPLE SPORT AND ITS VEGETATIVE PROGENY. Mich.
       Agr. Expt. Sta. Tech. Bul. 161, 14 pp., illus.
(3) ———
    1943. STUDIES IN THE NATURE OF THE CLONAL VARIETY. III. PERMANENCE
       OF STRAIN AND OTHER DIFFERENCES IN TREE SIZE IN THE MONT-
       MORENCY CHERRY. Mich. Agr. Expt. Sta. Tech. Bul. 186, 20 pp.,
       illus.
(4) ———
    1944. A STUDY OF THE SWEET-AND-SOUR APPLE CHIMERA AND ITS CLONAL
       SIGNIFICANCE. Jour. Agr. Res. 68: 383–394, illus.
(5) ———and Baten, W. D.
    1942. STUDIES IN THE NATURE OF THE CLONAL VARIETY. II. SELECTION
       WITHIN A PERICLINAL CHIMERA. Mich. Agr. Expt. Sta. Tech. Bul.
       179, 48 pp., illus.
(6) ———CRIST, J. W., and GIBSON, R. E.
    1933. SOMATIC SEGREGATION IN A SECTORIAL CHIMERA OF THE BARTLETT
       PEAR. Jour. Agr. Res. 46: 1047–1057.
(7) ———TOENJES, W., and GIEFEL, M.
    1948. SEGREGATION IN RUSSETED SPORTS OF THE GRIMES APPLE. Jour. Agr.
       Res. 76: 225–229, illus.
(8) NEWCOMER, E. H.
    1943. STUDIES IN THE NATURE OF THE CLONAL VARIETY. IV. CYTOLOGI-
       CAL STUDIES OF BUD SPORTS OF MCINTOSH, STARK AND BALDWIN
       APPLES. Mich. Agr. Expt. Sta. Tech. Bul. 187, 23 pp., illus.

SEGREGATION IN A RADIALLY UNSYMMETRICAL SPORT OF THE CANADA RED APPLE [1]

By V. R. GARDNER, *director, Michigan Agricultural Experiment Station,* W. TOEN-JES, *superintendent, Graham Horticultural Experiment Station,* and M. GIEFEL, *research assistant, Michigan Agricultural Experiment Station*

INTRODUCTION

Bud sports of fruits that differ from their parents in size are not of common occurrence. They appear now and then, however. In 1936 Shamel and Pomeroy (8)[2] stated that they had found references to 30 large-fruited and 2 small-fruited apple sports in their survey of the literature that had appeared up to that time, while they had found 280 references to color variations. Size variations in the grape appear to be relatively more numerous, Shamel and Pomeroy (8) listing 56 of this type out of a total of 78 affecting all characters of fruit. A few of the large-fruited forms possess commercial value and have been introduced into cultivation. Among these may be mentioned the King (3, pp. 324-325) and Lancaster (1) grapes, the Coates (9, 7) prune, sports of the Concord, Worden, and Agen varieties, re-respectively, and the Carapinheira (4) pear.

Apparently the fruits of most of these sports are simply larger or smaller, as the case may be, than the normal forms from which they have sprung, resembling them closely in shape, color, flavor, season of maturity, and other characteristics. A few, on the other hand, show a tendency to be irregular or offtype in shape. This irregularity of the few is most likely to take the form of a lack of radial symmetry, evidenced by more or less prominent longitudinal ridges and grooves. Most of the references in the literature to this particular type of variant have been to citrus species, and such terms as "ribbed," "corrugated," and "offtype" have been applied to them. These large-fruited, irregular or offtype sports have been dismissed as curiosities or monstrosities and very few have been propagated.

It has been noted that at least in some instances (e. g., the Karr strain of the Bartlett pear (10) limb sports which produce large-sized fruits likewise have leaves larger than those characteristic of the parent variety and sometimes their spurs, shoots, and branches are thicker and more stocky. There is evidence that certain of these giant forms are associated with a tetraploid condition of the chromosomes or other chromosomal abnormalities (5).

---

[2] Italic numbers in parentheses refer to Literture Cited, p. 255.

787555—48——3

MATERIALS AND OBJECTIVE

·Incident to the study of bud sports that has been under way at the Michigan station for many years a considerable number of large-fruited and of small-fruited variants have come under observation. These observations have included such well known varieties as the Bartlett pear, the Wealthy, Duchess, Rhode Island Greening, Grimes, Jonathan, Northern Spy, Baldwin, Delicious, Fameuse, Winesap, and Canada Red apples, and the Hyslop crab apple. Oddly enough, in view of the infrequent references to them in the literature more large-fruited forms have been found that are ribbed or corrugated and that are radially unsymmetrical than of normally shaped radially symmetrical forms. These rogues occur more frequently in certain varieties, e. g., McIntosh and Rhode Island Greening, than in others. Most of these unsymmetrical large- or small-sized sports are so extreme in their irregularity that they are spoken of by the fruit grower as "offtypes" or "rogues." There is reason to believe that they are in reality of more frequent occurrence than casual orchard inspection would indicate, for most fruit growers, upon finding such limb sports or occasionally whole tree sports, promptly remove them or graft them over to something whose fruit is more salable.

One of these aberrant forms, a sport of the Canada Red (Steele Red) apple, was brought to the writers' attention in the early thirties. There were 40 to 50 top-grafted trees of this strain in an orchard near South Haven, Mich., the scions for all of which had been cut from a single tree of that variety some 12 or 15 years earlier. The parent tree had been cut down in the meantime. This entire group of trees bore fruits showing great variation in size and shape. Some fruits were normal in size, some were below normal, and still more above. A few especially of the normal-sized ones, were regular and symmetrical in shape; most of them, especially the undersized and oversized specimens, were irregular and radially unsymmetrical (fig. 1). The trees of the entire group were not examined thoroughly and systematically limb by limb, to determine whether there was a single branch somewhere that bore exclusively undersized or normal or oversized fruits or exclusively regular- or irregular-shaped fruits. However, no such branches were observed. There seemed to be a more or less random distribution of fruits of all sizes and shapes on each and every tree and likewise on each and every top-grafted limb (5 to 10 per tree). Here and there in practically every tree single specimens were noted that were large-sized and radially symmetrical or practically so. It is emphasized, however, that these were isolated and scattered, not segregated in groups.

The lack of radial symmetry of these fruits was extreme, and yet it followed a pattern or series of patterns. Each radially unsymmetrical fruit presented more or less the appearance of a composite of 1 to 4 segments (corresponding to carpels) of large size combined with 4 to 1 segments of normal or of small size; the large radial segments might be adjacent to each other or 2 such segments might have a normal-sized or a small segment between them. Theoretically 39 combinations of such size elements are possible in a 5-carpelled fruit. To the eye some of these combinatins resemble each other rather closely; some are much more extreme than others. Actually how many of them occurred or

FIGURE 1.—End and side views of four typical fruits of Canada Red selection No. 526.   All are of giant size for this variety, about 1½ times the size of the normal fruit, and all are irregular in shape and radially unsymmetrical.

the relative frequency of certain of them is not known.   Furthermore, normal unequal growth of the 5 segments of a radially symmetrical fruit is great enough to make exact identification of all the classes of irregularity impracticable, if not totally impossible.   Suffice it to say that a large percentage of the fruits were radially unsymmetrical and more of them distinctly so than obscurely so.

The fact that some of the fruits borne by these Canada Red trees were less irregular and unsymmetrical to the eye than others, and more especially that a few appeared to be entirely regular and symmetrical,[3] suggested the possibility through scion selection and propagation of segregating out from the parent stock a line or strain uniform for large size—i. e., with all five segments or carpels of the fruit of large size— and consequently radially symmetrical.   Of course the same possibility was presented of segregating out a uniform normal-sized, radially symmetrical and a uniform small-sized, radially symmetrical strain.

___

[3] Assuming random or chance combinations and juxtapositions of large, normal, and small sizes to make up the 5 segments or carpels, if all basic elements are present and have equal opportunities to combine in one way as well as another, 1 fruit out of every 81 could be expected to be regular and symmetrical and 1 out of every 243 could be expected to be large sized, regular, and symmetrical.

Scions (numbered as selections 526 to 532, inclusive) were taken from seven of the Canada Red top grafts and used for production of whole trees and top grafts at the Graham Horticultural Experiment Station. Sixteen top grafts and 21 whole trees of these selections were grown to fruiting age.

Fruits were first produced by some of the top grafts in 1937. Altogether 10 years' fruiting records were obtained at the time of the preparation of this report, though in some seasons the crops were so small because of spring frost damage or other factors that records consisted only of brief notes. However, in those years when any of the top grafts or whole trees produced a considerable number of fruits each fruit was weighed to the closest 10 gm. and was classified as regular and symmetrical in form or as irregular and radially unsymmetrical. In some seasons only certain of the top grafts or certain of the whole trees bore enough fruits to make such detailed records seem worth while. Enough records were obtained, however, or what was regarded as representative tree performance to make certain comparisons.

PRESENTATION OF DATA

The performance of the individual top grafts and the whole trees of the several selections can best be presented in the form of a series of frequency distribution tables showing fruit sizes as determined by weight and the classification of the same fruits as regular or irregular in shape. In the matter of weight exact data were obtained; classification as to regularity in form involved judgement, and different individuals were employed for this work in different seasons. Furthermore, as stated earlier, fruits that obviously should be and were classified as regular were not in fact absolutely regular; the cross sections of few of them would be perfect circles. Certainly out of every hundred normal ungraded fruits of almost any variety, taking them just as they are harvested, there are likely to be a dozen that are more or less irregular and unsymmetrical, perhaps because of insect or fungus injury, abortion of seeds in one or more of the seed cavities, or other factors. In some seasons the percentages of such normally irregular fruits were small; in others they might run as high as 15 to 20. Doubtless, some specimens that should have been classified as irregular were actually classified as regular; probably a still larger number were classified as irregular that should have been classified as regular.

Fruits of selection No. 587 were weighed and classified as to regularity and used as a check on selections 526 to 532. Scions for selection 587 had been obtained from Connecticut and were supposed to be of an especially large-sized strain of the variety. Actually this supposedly large-sized strain proved to be normal for the variety. It will be noted that in 1946 a considerable number of the fruits of this normal stock were classified as irregular in shape (table 1). Those top grafts and trees of selections 526 to 532 that did not produce relatively more irregular-shaped fruits than the trees of No. 587 may be regarded as producing normal-shaped fruits.

TABLE 1.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 587 (check), seasons of 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree—										1944, tree—										1946; tree—									
	7-6-B R	I	7-6-D R	I	6-I R	I	7-I R	I	0-J R	I	7-6-B R	I	7-6-D R	I	6-I R	I	7-I R	I	6-J R	I	7-6-B R	I	7-6-D R	I	6-I R	I	7-I R	I	6-J R	I
20																														1
30											1																			2
40	4										4														4				3	5
50	7										4	1																	2	2
60	8	1			2						8	1													8				15	13
70	9		1		5		1				28		1												28		1		37	3
80	1	1		1	5		2				48		4												60		2		39	5
90	1	1	1	2	7		7		2		71		7		3				1						58		7		70	7
100	1		1	1	9		12		2		53		14	1	5				1						64		12		85	3
110	1		2	1	8		6		6		51		14		10		7		7						46		16		82	2
120			2		9		10		8		36		15		4		14		15	1					33		30	1	63	1
130			2		7				7		18		13		6		9		14						33		35	3	47	1
140			2		4		3		2		7		5		9		11		14						11		40		30	
150			1		1				7		3		4				9		20						4		34	1	15	
160									1				1		5		2		11								24	2	9	
170													2		2		4		4								9	2	3	
180											1				3		6		6								5	1	4	
190											1						3										6			
200											1						2										1	2		
210																	1													
220																														
230																														
Total	32	3	13	5	60		41		35		334	3	80	1	47		84		100	1					346		210	13	504	45
Mean	105	100	124	104	103		105		116		97	70	116	110	138		144		147	140					99		89	75	116	80
Mean of all (weighted)	108										116										104									

TABLE 2.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 526, seasons of 1943, 1944, and 1946*

[R=regular in shape; I=Irregular]

Weight (grams)	1943; tree—										1944; tree—										1946; tree—									
	7-6-B		7-6-D		1-G		1-H		1-J		7-6-B		7-6-D		1-G		1-H		1-J		7-6-B		7-6-D		1-G		1-H		1-J	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
20												1														4				
30												3														3				2
40		3										10		1											1	1				1
50		6										17		1											2	1				1
60		24		4		2		1				38		1											2	7		1	1	1
70		34		4		4		1		3		33		2		5									2	6				1
80	2	20		5		3		2	1	7		30		1	1			1							4	16	1	1	1	3
90		10		2		3		2	1	11		16			1	2	1	3							5	7		3	2	20
100		4		1		2		3		7		13		9	3	3		5							10	13	1	9	1	25
110						1		4		8		8		5	2	2	3	9	1						12	18	3	19	2	17
120						1		8		3				4	2	1	5	13							5	8	3	25	4	14
130								6						1	1	1	9	14							11	14	9	31	4	7
140								4						1		1	13	10							5	5	9	15	2	8
150								4						1	3	3	14	10	3						4	5	19	14	2	3
160								1							1	1	10	7							4	3	25	8	2	
170															2	2	10	5							5	3	30	7		1
180																	7	5									31	4	2	1
190								1			1						5	4	1								15	1	1	
200											1						5	2									14	2		
210																	4	4									8			
220																	2										7			
230																			2								4	1		
240																	1	1									1	2		
250																											2			
260																	1													
270																											1			
280																														
290																											1			
300																														
Total	2	135		18	16	94	35	123	2	39	191	88	5	56	10	21	96	180	7	114					68	103	186	165	26	105
Mean	80	86		88	94	90	35	123	65	75	191	88	78	93	165	114	96	180		114					112	93	186	165	142	127
Weighted mean					90										116										133					

TABLE 3.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 527, seasons of 1943, 1944, and 1945*

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree—										1944; tree—										1946; tree—									
	7-6-B		2-G		2-H		2-I		2-J		7-6-B		2-G		2-H		2-I		2-J		7-6-B		2-G		2-H		2-I		2-J	
	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I	R	I
30																									6					
40			2																						15					
50			5																						18	3				
60			11								1											1	1		25	1	1	1		
70	2		21			1	1		3		4														31		1	1	1	
80	3		21	1	1		2		10		3						2						3		26	2	7	1	2	
90			10	1			6		13		9	1	1				2					1	6		11	5	4	7	7	
100	8		6	1			5		13		13						3					3	13		25	2	12	1	9	
110	2		3	1	1		2		14		14						5					6	21	2	26	1	14	1	4	
120	1						4	2	3		25		4				2					13	47	1	11		32		7	1
130									5		16		1				1					21	56		25	1	24	1	3	1
140											12		1				3					47	51		16		35	2	2	
150											12		1									56	32		6		32	1		
160											5		1				2					51	36		8		27	2		
170											6											32	17		3		19			
180											1		2									36			1		11			
190													2									17	2	2	1		5	1		
200													1											6			6			
210																								7						
220																								3			2	1	2	
230																								1			1			
240																														
250																														
260																														
Total	16		79	4	2	1	22		48		121		16				25					304	7	254	17	239	16	37	4	
Mean	105		106	123	80	60	102		84		111		156				154					147	141	97	88	163	142	149	127	
Weighted mean	99										122										135									

TABLE 4.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 528, seasons of 1941, 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	1941 7-6-B R	1941 7-6-B I	1941 7-6-D R	1941 7-6-D I	1941 3-G R	1941 3-G I	1941 3-H R	1941 3-H I	1943 7-6-B R	1943 7-6-B I	1943 7-6-D R	1943 7-6-D I	1943 3-G R	1943 3-G I	1943 3-H R	1943 3-H I	1944 7-6-B R	1944 7-6-B I	1944 7-6-D R	1944 7-6-D I	1944 3-G R	1944 3-G I	1944 3-H R	1944 3-H I	1946 7-6-B R	1946 7-6-B I	1946 7-6-D R	1946 7-6-D I	1946 3-G R	1946 3-G I	1946 3-H R	1946 3-H I
40	4																		4													
50	1								1										4										7	1		
60	5								12	1									9										13	2	1	
70	6								25	3	3	1							18	1									37	4	2	1
80	8		2						58		2	3	2				6		31	2	2								57	9	8	2
90	15	1	3						65		8	2	2				8	1	50		2								49	5	13	3
100	14	1	3						56	1	2	3	2				15		98	1	1								62	5	27	3
110	16		1	1					44	1	8	6	3				21		80	1	5								36		17	2
120	8		12						18		13	3					18		61	1	1	1							11	1	10	1
130	10		7						9	2	16	1					16		33	1	5								6		10	
140	11		15						4		11						14		29		7										7	
150	13		11						1		6						5		11		6								2		3	
160	3		10								6						5		3		8										1	
170	5		11								3						4		1		8											
180			13														1				4											
190			3								1								1		4	1										
200			5																		3											
210																					2											
220																					1											
Total	104	3	96	1					293	8	73	7	10				124	1	433	7	57	2							283	39	97	11
Mean	144	140	151	120		147			90	97	122	97	97				114	90	106	103	147	175							89	82	118	109
Weighted average						147								97								111									97	

TABLE 5.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red Selection No. 529, seasons of 1943, 1944, and 1946*

[R=Regular in shape; I=irregular]

Columns 1–10 = 1943; tree — Columns 11–20 = 1944; tree — Columns 21–30 = 1946; tree

Weight (grams)	7-6-B R	7-6-B I	7-6-D R	7-6-D I	4-G R	4-G I	4-I R	4-I I	4-J R	4-J I	7-6-B R	7-6-B I	7-6-D R	7-6-D I	4-G R	4-G I	4-I R	4-I I	4-J R	4-J I	7-6-B R	7-6-B I	7-6-D R	7-6-D I	4-G R	4-G I	4-I R	4-I I	4-J R	4-J I
40																														
50																									2					
60											1		3												6				1	
70							2				2		6												15		2		2	
80			1				2				8		10												23	1	4		3	
90	2						4				13		16												39	3	7	1	9	1
100	9		6				2				24		13	1											38	3	7	2	14	1
110	1		4				3				14		27	1											44	2	20	4	36	2
120	1		9				1				21		16												31		30	3	35	2
130	3		9								15		30												17		36	2	48	2
140	2		11								5		18												17		38	6	38	3
150	3		6								3		15	1					1						3		34	3	47	1
160			5								2		10						2						5		10	3	36	1
170			2										8						1						2		14	3	26	1
180			4										5												1		7	1	15	
190													3						1								4		8	
200													1														2		8	
210																											1		4	
220																														
230																													1	
240																														
250																														
260																														
270																														
280																														
Total	21		57				14				108		181	3					6						243	9	216	28	331	14
Mean	120		134				95				100		107	97					142						95	91	146	139	150	124
Weighted average	125										105										131									

TABLE 6.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 530, seasons of 1941, 1943, and 1944*

[R=regular in shape; I=irregular]

Weight (grams)	1941; tree— 7-6-B R	7-6-B I	7-6-D R	7-6-D I	1943; tree— 7-6-B R	7-6-B I	7-6-D R	7-6-D I	1944; tree— 7-6-B R	7-6-B I	7-6-D R	7-6-D I
40									1			
50									3		1	
60							3		10		3	
70			2		1		2		16		8	
80			4		1		15		37		15	
90			2		3		14	1	44		23	
100	2		5		6	1	18		52		27	
110	3		12		13	1	14		50		30	
120	5	1	8	1	19	1	10		31		23	
130	1		10		17		7		18		18	
140	2			1	13		2		9		11	
150	2				6				4		7	
160	7		10		1				3		2	
170	3		6		3				1			
180	1		1		2							
190					15		2					
200	1		1	1	23		14					
210	3			2	27		18					
220	2		2		30		11					2
Total	32		64		79	3	85	1	279	3	168	2
Mean	152		137		95	117	109	110	101	1	101	90
Weighted average	142				102				101			

TABLE No. 7.—*Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red Selection No. 591, seasons of 1943, 1944, and 1946*

[R=regular in shape; I=irregular]

Weight (grams)	'43 7-6-B R	'43 7-6-B I	'43 7-6-D R	'43 7-6-D I	'43 5-G R	'43 5-G I	'43 5-H R	'43 5-H I	'43 5-I R	'43 5-I I	'43 5-J R	'43 5-J I	'44 7-6-B R	'44 7-6-B I	'44 7-6-D R	'44 7-6-D I	'44 5-G R	'44 5-G I	'44 5-H R	'44 5-H I	'44 5-I R	'44 5-I I	'44 5-J R	'44 5-J I	'46 5-G R	'46 5-G I	'46 5-H R	'46 5-H I	'46 5-I R	'46 5-I I	'46 5-J R	'46 5-J I
20																																1
30																																3
40																										1					7	8
50		1														3										1			1		6	10
60		2				1	4	2						2		5										5			6		14	6
70		4	1	1	1	3	6	4						3		10	1	3								14	2		16		14	7
80		2	1	1	1	4	4	1	1					7		8	1	7								37	4		26		8	8
90		2	1	9	1	2	4	4	1			1		17		10	1	9						1		43	2	1	39	1	6	4
100	1	1	2	11		10	3	1	1					21		10	2	7					1			59	4	2	43	2	2	1
110		3	1	18		11	3	1			1			3		8	1	4			1		1		2	66	7	2	40	2	2	2
120	1		1	14		5	3				1			4		7	1	4			1			2	2	56	3	3	32	3		1
130			1	11		6					1			1		1	1	3			1				1	34	7		30		1	
140						5								6		2	1	5			1		1	1	3	27	3		14	2	2	
150			1	2		5								1		1		4							6	27	1		10			
160														4		1		1							3	14	2		4	1	1	
170																									2	13	3		4	1		
180																									2	4	5		5			
190																									1	2	1					
200																									1		1		1			
210																																
Total	1	15	9	67	3	52	27	13		3	3	1		69		77	9	47			4		2	5	24	403	45		271	12	63	51
Mean	120	91	75	91	90	99	76	61		73	113	60		96		89	99	119			120		90	122	138	115	112		119	122	93	63
Weighted average							93											100											111			

TABLE No. 8.—Frequency distribution table showing sizes and regularity of fruits borne by top grafts and trees of Canada Red selection No. 532, seasons of 1943, 1944, and 1946

[R=regular in shape; I=irregular]

Weight (grams)	1943; tree— 7-6-D R	I	6-G R	I	6-H R	I	1944; tree— 7-6-D R	I	6-G R	I	6-H R	I	1946; tree— 7-6-D R	I	6-G R	I	6-H R	I
30																		1
40															4			1
50															6	2		2
60							1								11	3	1	
70			1			1									19	1	2	2
80	1			1			1								31	3	7	4
90			2				2								52	2	6	5
100	1		1				4		1		1				68	3	35	5
110							2		1		6				52	3	36	10
120					2		1		6		8				37	3	37	7
130							1				7				29	2	18	10
140			1				1		3		3				22	1	27	6
150							2		1		9				8	1	15	1
160							2		2		7				4		13	3
170							1		3		6				1		8	1
180									2		1						4	1
190									4						1		2	
200									2		1						1	
210									1		1						1	
Total	2		5	1	2	1	18		26		50				345	24	213	59
Mean	90		98	80	120	70	118		156		143				103	96	123	113
Weighted average	96						142						110					

Some variation in size of fruit was due to environmental influences. Thus 1946 was a year of drought and in general fruit size was unfavorably affected. Of even greater importance in this study was the influence of relative size of crop borne by certain trees or top grafts, heavy production being responsible for smaller than normal size of individual fruits. No attempt was made to classify the crop of each and every tree and top graft each year as light, medium, or heavy and there is consequently no indication in the tables as to whether any one year's record for a tree is for a heavy or light crop year. Some of the apparent inconsistencies in the tables are due to this factor. Because of the larger crops borne in 1944 and 1946 the data for those years are to be regarded as more representative than those presented for earlier seasons. The records for those 2 years, however, must be interpreted only after giving due consideration to the factor of relative load of fruit. Thus the large sizes of the fruits of selection 532 trees 6–G and 6–H in 1944 (table 8) are obviously associated with light cropping and do not indicate the true fruit size potentialities of the two trees. Mean weights of 116 and 104 gm. (table 1) respectively, for the seasons of 1944 and 1946 may be considered representative for young trees of this variety under a clean culture-cover crop system of soil management.

It will be noted that on the whole the trees and top grafts of all 7 selections (526 to 532) produced fruits that in average size were not greatly different from those of the check trees (No. 587, table 1). Closer examination of the data, however, shows some notable exceptions. Thus tree 5–J and top grafts 7–6–B and 7–6–D of selection 531

(table 7) have consistently produced rather small-sized fruits, while trees 5–G and 5–H of the same selection have consistently produced fruits of about normal size.   Tree 4–J of selection 529 (table 5), 2–I of selection 527 (table 3), and 1–H of selection 526 (table 2), have consistently borne large fruits, while trees 4–G, 2–G, and top graft 7–6–B of the same respective selections have consistently borne small fruits. In these particular instances the crops were near enough the same size to make comparisons valid.   It would appear that both small-sized and large-sized strains have been segregated out of the original stock, strains that are as uniform for their large size or their small size as the normal strain (No. 587).

In respect to regularity in shape, the top grafts and whole trees on the whole have produced a higher percentage of regular, radially symmetrical fruit than the parent stock from which they were propagated.   However, certain top grafts and likewise certain trees (e. g., top grafts 7–6–B, 7–6–D, and trees 1–G and 1–J of selection 526 (table 2)) have borne irregular fruits almost exclusively, while certain other top grafts and trees have borne regular fruits almost exclusively. Regularity in shape may be associated with small size as in tree 5–J of selection 531 (table 7 and fig. 2), with normal size as in tree 6–H of
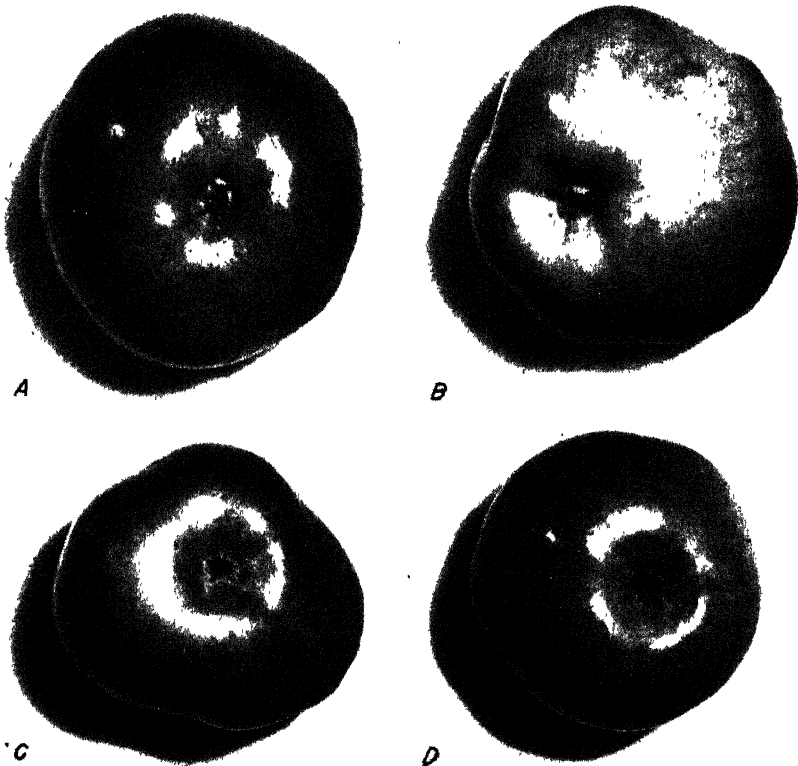
FIGURE 2.—Fruits from tree 5–J of selection 531: *A*, Normal-sized, symmetrical specimen; *B*, normal-sized, radially unsymmetrical specimen; *C* and *D*, small-sized, radially unsymmetrical specimens.

selection 532 (table 8), or with large size as in tree 4–I of selection 529 (table 5). Similarly, regularity in shape may be combined with small size as in tree 4–G of selection 529, with normal size as in top graft 7–6–D of selection 528 (table 4), or with large size as in tree 1–H of selection 526 (fig. 3). From a practical standpoint it is this last
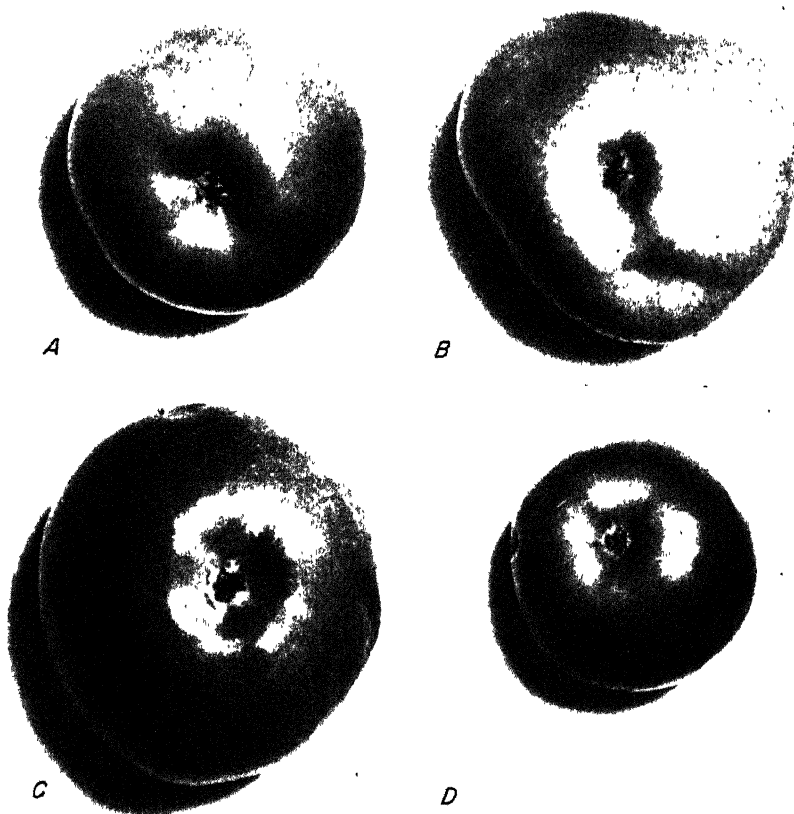
FIGURE 3.—Fruits from selections 587, 529, and 526: *A*, Normal-sized regular fruit from selection 587; *B*, large-sized, radially unsymmetrical specimen of selection 529 from tree 4–I; *C*, a typical large-sized regular fruit of selection 526 from tree 1–H; *D*, small-sized, regular segregate of selection 529 from top graft 7–6–B.

combination for which the apple industry is looking. It appears as though several of the trees and top grafts possess this combination— tree 1–H of selection 526 being perhaps the best representative.

DISCUSSION

The irregular, radially unsymmetrical sports of the Canada Red apple described in this paper are evidently comparable in certain respects to recorded sports of the bizzarria (*11*), Buckeye Navel (*6*), and Thompson Navel (*2*) oranges. These citrus sports are variously classified as sectorial or periclinal chimeras or as due to genic insta-

bility. The descriptions of most of them indicate that they are ever-sporting forms, through at least in the case of the bizzarria *(11)* orange fairly stable segregates have appeared. The Canada Red apple sport would seem to be a sectorial chimera. The two interesting things about it are that: (1) apparently this particular type of sport has not been recorded as occurring in the apple, and (2) out of it has been segregated new comparatively stable strains, at least one of which seems to be promising commercially.

The study suggests that in the case of certain tree fruits and perhaps some of the long-lived, slow-growing ornamentals for which many years are required to obtain a new seed generation, the isolation and propagation of desirable segregates from naturally occurring sports may afford a practicable method of developing new varieties.

SUMMARY

An irregular, radially unsymmetrical bud sport of the Canada Red apple is described, together with its vegetative progeny.

Some of the top grafts and whole trees propagated from this sport closely resemble the parent form. Others show segregation of parental characteristics. Some of these segregates are characterized by a degree of uniformity more or less comparable to that of the parent Canada Red. One of them appears to be worthy of introduction as a new or improved strain of that variety.

LITERATURE CITED

(1) CAMP, C. B.
    1912. NEW VARIETIES OF 'GRAPES. Nebr. State Hort. Soc. Ann. Rpt.
        43: 151–154.
(2) FROST, H. B.
    1926. POLYEMBRYONY, HETEROZYGOSIS AND CHIMERAS IN CITRUS. Hilgardia 1: [365]–402, illus.
(3) HEDRICK, U. P.
    1908. THE GRAPES OF NEW YORK. 564 pp., illus. Albany, N. Y.
(4) NATIVIDADE, J. V.
    1939. MUTAÇÕES SOMÁTICAS EM VARIEDADES PORTUGUESAS DE POMÓIDEAS.
        (BUD MUTATIONS IN PORTUGUESE PEAR AND APPLE VARIETIES.)
        Agronomia Lusitana 1: 7–21, illus. [In Portuguese. English summary, pp. 19–20.]
(5) NEWCOMER, E. H.
    1943. STUDIES IN THE NATURE OF THE CLONAL VARIETY. IV. CYTOLOGICAL STUDIES OF BUD SPORTS OF MCINTOSH, STARK AND BALDWIN APPLES. Mich. Agr. Expt. Sta. Tech. Bul. 187, 23 pp., illus.
(6) POPE, W. T.
    1934 CITRUS CULTURE IN HAWAII. Hawaii Agr. Expt. Sta. Bul. 71, 37 pp., illus.
(7) SHAMEL, A. D.
    1919. ORIGIN OF A NEW AND IMPROVED FRENCH PRUNE VARIETY. Jour. Hered. 10: 339–343, illus.
(8) ———— and POMEROY, C. S.
    1936. BUD MUTATIONS IN HORTICULTURAL CROPS. Jour. Hered. 27: 487–494, illus.
(9) ———— POMEROY, C. S., CARYL, R. E., and HARMON, F. N. 1933. BUD VARIATIONS IN THE AGEN PRUNE. Jour Hered. 24: 289–292, illus.
(10) ———— Pomeroy, C. S. and Harmon, F. N.
    1931. BUD VARIATION IN BARTLETT PEAR TREES. Jour. Hered. 22: 81–89, illus.
(11) TANAKA, T.
    1927. BIZZARRIA—A CLEAR CASE OF PERICLINAL CHIMERA. Jour. Genet. 18: [77]–85, illus.

CONTRIBUTION OF INBRED LINES TO THE RESISTANCE OF HYBRID DENT CORN TO LARVAE OF THE EARLY SUMMER GENERATION OF THE EUROPEAN CORN BORER [1]

By L. H. PATCH, *associate entomologist*, and RAY T. EVERLY, *associate entomologist, Division of Cereal and Forage Insect Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture* [2]

INTRODUCTION

In a previous publication [3] certain inbred lines of dent corn (*Zea mays* L.) were shown to be resistant, partially resistant, or susceptible to the survival of larvae of the early summer generation of the European corn borer (*Pyrausta nubilalis* (Hbn.)), and to be able to transmit this resistance or susceptibility to their hybrids. Since there were fewer borers from eggs that hatched on corn in a less advanced stage of growth than from eggs that hatched on corn in a more advanced stage, the population of borers to be expected in each strain was predicted on the basis of the regression of number of borers per plant on silking date.

The inbred lines or corn hybrids that consistently contained fewer than the predicted number of borers were classed as inherently borer-resistant. One experiment involved different combinations of these lines in single-cross hybrids. From borer populations in resistant single crosses averaging 39 percent less than the predicted number of borers, the populations increased to 2 percent more than the predicted number in single crosses made up entirely of partially resistant lines, and to 58 percent more than the predicted number in single crosses involving only susceptible lines. The cumulative effect of multiple factors in inbred lines on borer resistance in hybrids was clearly indicated.

The purpose of the work herein described was to determine whether or not the quantitative effects of the inbred lines in the single crosses were the same as those with double-cross hybrids involving, with two exceptions, the same lines used in the single crosses. Incidental to this study, the possibility of complementary or modifying factors for borer resistance was considered.

STRAINS TESTED

Single-cross hybrids were tested in 1939 and double crosses in 1941, at Toledo, Ohio. The 6 possible single-cross combinations of resistant, partially resistant, and susceptible inbred lines were each

[3] PATCH, L. H., HOLBERT, J. R., and EVERLY, R. T. STRAINS OF FIELD CORN RESISTANT TO THE SURVIVAL OF THE EUROPEAN CORN BORER. U. S. Dept. Agr. Tech. Bul. 823, 22 pp. 1942.

Journal of Agricultural Research,
Washington, D. C.
(257)

Vol. 76, No. 11
June 1, 1948
Key No. K-344

represented by 3 to 10 crosses. The crosses involved the resistant lines Ia. L317, Ill. R4, Mich. 77, and Mich. 106, the partially resistant lines Ill. Hy, Ind. TR, Ia. I205, and U. S. 540, and the susceptible lines Ill. A, Ill. 90, Ind. WF9, and U. S. 187–2. Lines L317, R4, Hy, and A in all combinations and crosses on lines TR, I205, 540, 90, WF9, and 187–2 were tested. . In addition, line R4 crosses on 77 and 106, 77 on 106, and 540 on 77, 106, and I205 were tested, making a total of 36 single crosses.

Each of the 15 possible double-cross combinations of resistant, partially resistant, and susceptible inbred lines was represented, with one exception, by 4 double crosses. In order that the combinations made up entirely of resistant or partially resistant lines be represented by more than one double cross, it was necessary to use at least 1 inbred line in addition to the 4 lines involved in the resistant and partially resistant groups of the single crosses. Hence, resistant Wis. CC5 and partially resistant Wis. CC1 were also used in the pedigree of the double crosses. In earlier experiments [4] these lines had about the same effect on borer survival as the other lines in their respective groups, and, as shown in figure 1, a comparison of the single crosses with the double crosses disclosed no significant changes resulting from the use of these lines in the pedigrees of the double crosses. In the combinations made up entirely of susceptible lines, however, another line showing consistent susceptibility to the borer was not available at the time of the experiment. Hence, double cross (A×90) × (WF9×187–2) was entered 4 times under that combination.

The pedigrees of the 60 double crosses involved the following single crosses: 16 made up entirely of single crosses used in the foregoing test of single crosses; 13 made up with only 1 of the 2 single crosses involving either CC1 crossed on R4, Hy, or A, or CC5 crossed on R4 or A; and 31 made up of 2 single crosses, at least 1 of which was not used in the test of single crosses although they involved the same inbred lines as those crosses. The 31 double crosses involved single crosses of 106 on L317, Hy, and A, of TR on I205, 540, and 90, of WF9 on TR, I205, 540, 90, and 187–2, and of 187–2 on I205 and 540.

The hybrids were designated according to the borer reaction of the inbreds involved. For example, a single cross involving 1 resistant, no partially resistant, and 1 susceptible inbred was designated as a 1–0–1 hybrid combination; and a cross made up of no resistant, 1 partially resistant, and 1 susceptible inbred as a 0–1–1 combination. In double crosses a cross involving 2 resistant, 1 partially resistant, and 1 susceptible inbred was designated as a 2–1–1 hybrid combination.

METHODS AND ANALYSIS OF DATA

The single crosses were grown in 2-hill plots of 3 plants per hill with 7 replications, and the double crosses in 1-hill plots of 3 plants per hill with 8 replications. The hills were 42 inches apart each way. Each plant was infested by hand with 4 egg masses, or about 80 eggs, before the tassels became a factor in borer survival, and each was tagged with the date of silking. All plants were dissected during the last 2 weeks of August to determine the number of borers surviving. The differences in the number of eggs laid naturally on the different hybrids were so small that they were not considered as a factor in the study.

---

[4] See footnote 3, p. 257.

The first step in the analysis of the data was to eliminate the effect of differences in maturity among the hybrids on the mean number of borers per plant in each hybrid, by use of a regression coefficient determined from previous experiments.   The decrease in the mean
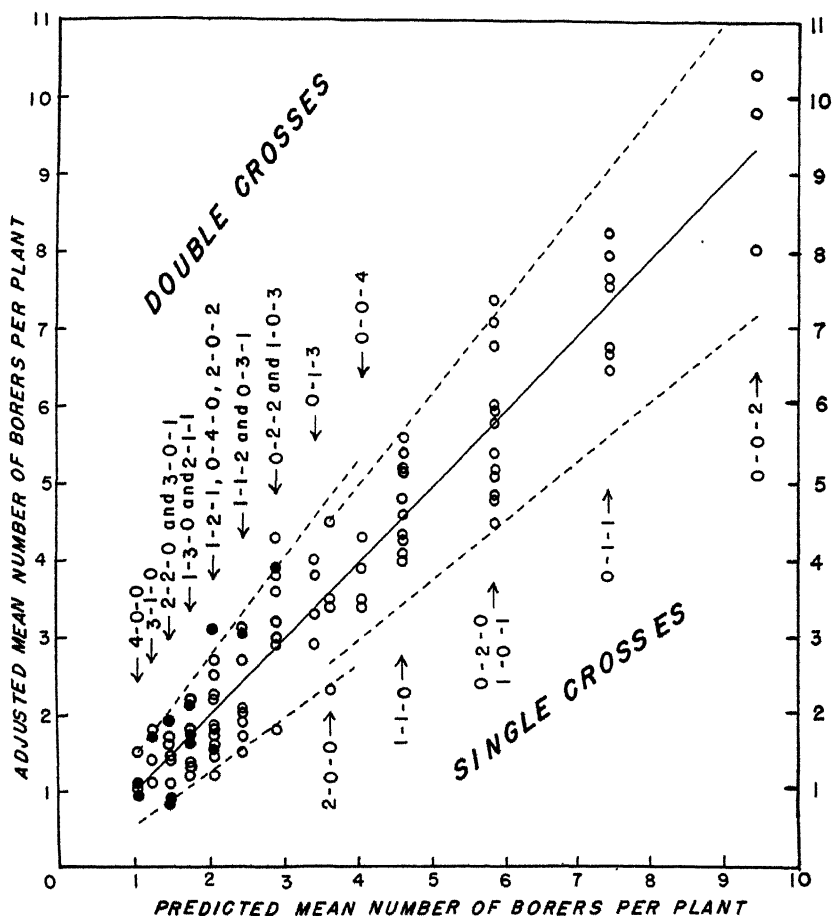
FIGURE 1.—Adjusted mean plotted against predicted mean number of European corn borers per plant in single-cross and double-cross combinations of resistant, partially resistant, and susceptible inbred lines of dent corn.   The first, second, and third figures in each set indicate, respectively, the number of resistant, partially resistant, and susceptible inbreds in the different crosses.   Open circles indicate different combinations of these lines.   Solid circles indicate double crosses in which Wis. CC5 and Wis. CC1 were used.   After variability due to replication is removed, 95 percent of the hybrids would be expected to fall within the dotted lines.

number of borers for each day later in silking may be closely estimated by multiplying the mean number of borers per plant in the experiment by 0.043.[5]   For each day earlier or later than July 23, the average

[5] PATCH, L. H., and EVERLY, R. T.   RESISTANCE OF DENT CORN INBRED LINES TO SURVIVAL OF FIRST-GENERATION EUROPEAN CORN BORER LARVAE.   U. S. Dept. Agr. Tech. Bul. 893, 10 pp., illus.   1945.

silking date of the single crosses averaging 6.06 borers per plant, 0.26 was added to or subtracted from the number of borers observed per plant to obtain the adjusted mean. In a similar manner the means of the double crosses that averaged 2.20 borers per plant were adjusted.

The second step was to determine the decrease in borer population in hybrids involving different combinations of resistant, partially resistant, and susceptible lines, as compared with hybrids made up entirely of susceptible lines. In the single crosses the borer populations in combinations 0–0–2, 0–1–1, 0–2–0, 1–1–0, and 2–0–0, were known to decrease from a high to successively lower levels.[6] In the double crosses the borer populations in combinations 0–0–4, 0–1–3, 0–2–2, 0–3–1, 0–4–0, 1–3–0, 2–2–0, 3–1–0, and 4–0–0 would be expected to decrease from a high to successively lower levels if the effects of the inbred lines in double crosses were similar to their effects in single crosses. Certain balanced combinations, such as 1–2–1, 0–4–0, and 2–0–2, would be expected to contain the same level of borers except for the variation due to sampling errors.

TABLE 1.—*Adjusted and predicted mean numbers of European corn borers in groups of single-cross and double-cross combinations of borer-resistant, partially resistant, and susceptible inbred lines of dent corn*

SINGLE-CROSS COMBINATIONS, 1939

1	2	3	4	5	6	7
				Predicted number of borers per plant		
Prediction group No.	Combination [1]	Number of hybrids in combination	Adjusted mean of borers per plant	Arithmetical progression	Geometrical progression	
					Empirical estimates [2]	By method of least squares [4]
1	2–0–0	4	3.43	3.57	3.522	3.611
2	1–1–0	10	4.73	4.67	4.508	4.590
3	{1–0–1	8	5.81	5.77	5.77	5.834
	{0–2–0	4	5.55	5.77	5.77	5.834
4	0–1–1	7	7.16	6.87	7.386	7.415
5	0–0–2	3	9.38	7.97	9.454	9.424

DOUBLE-CROSS COMBINATIONS, 1941

1	2	3	4	5	6	7
1	4–0–0	4	1.13	0.97	1 013	1.040
2	3–1–0	4	1.50	1.22	1.196	1.232
3	{2–2–0	4	1.58	1.47	1.412	1.460
	{3–0–1	4	1.13	1.47	1.412	1.460
4	{1–3–0	4	1.55	1.72	1.668	1.730
	{2–1–1	4	1.75	1.72	1.668	1.730
5	{0–4–0	4	2.23	1.97	1.97	2.050
	{1–2–1	4	1.77	1.97	1.97	2.050
	{2–0–2	4	1.90	1.97	1.97	2.050
6	{0–3–1	4	2.35	2.22	2.327	2.429
	{1–1–2	4	2.13	2.22	2.327	2.429
7	{0–2–2	4	3.63	2.47	2.748	2.878
	{1–0–3	4	3.00	2.47	2.748	2.878
8	0–1–3	4	3.50	2.72	3.245	3.410
9	0–0–4	4 4	3.78	2.97	3.832	4 041

[1] Respective numerals indicate the number of resistant, partially resistant, and susceptible inbred lines involved.
[2] The progression ratio is 1.280 and 1.181 for the single and double crosses, respectively.
[3] The progression ratio is 1.271 and 1.185 for the single and double crosses, respectively.
[4] The hybrid (A × 90) × (WF9 × 187–2) was entered 4 times under combination 0–0–4.

[6] See table 11 of reference cited in footnote 3, p. 257.

Column 5 of table 1 shows an arithmetical progression with values that closely approximate the adjusted mean numbers of borers (column 4) of the low-numbered prediction groups, but diverge rather widely from the means of the high-numbered groups. On the other hand, the geometrical progression (column 6) closely approximates the numbers of borers in all the groups. Both the arithmetical and geometrical progressions were determined empirically as giving the best fit to the adjusted data.

For a more accurate determination of the geometrical progression giving the best fit to the data, the method of least squares was employed. The adjusted mean number of borers, Y, in each hybrid was used. With the empirical values of the geometrical progression given in column 6 designated as X, a value of Y' was estimated for each value of X on the basis of the regression of Y on X. The values of Y were paired with the value of X for the group in which they occurred. The values of Y' gave the geometrical progression shown in column 7 of the table. The regression of Y on Y' gave a coefficient of 1.0 borer in each case.

The third step in the analysis of the data was to plot (fig. 1) the adjusted mean number of borers per plant, Y, dissected from the individual hybrids against Y', the number predicted for the group in which they occurred. The regression line was drawn through the plotted points. Then, after the variability due to replication was deducted, the variability from plot to plot within hybrids was determined for 26 levels of borer population by grouping hybrids according to borer level. The data from the double and single crosses fell so closely along the same regression line that both lots were considered as one set. The standard error of the mean of samples of 24 plants containing an average of 1 borer per plant was found to be 0.24, and this value increased by linear regression to 1.46 for samples averaging 9.5 borers per plant. With twice the standard error of the mean of 42 plants for the single crosses and of 24 plants for the double crosses, limits were set off above and below the regression line in figure 1 within which 95 percent of the plotted points would be expected to lie.

EFFECTS OF INBRED LINES IN HYBRID COMBINATION

The combinations of single crosses made up entirely of susceptible, partially resistant, and resistant lines contained estimated averages of 9.42, 5.83, and 3.61 borers per plant, respectively, as compared with 4.04, 2.05, and 1.04 borers in the same combinations of double crosses. There were 61.9 percent as many borers in the partially resistant as in the susceptible combinations of single crosses compared with 50.7 percent in the double crosses. There were also 61.9 and 50.7 percent as many borers in the resistant as in the partially resistant combinations of single and double crosses, and 38.3 and 25.7 percent as many borers in the resistant as in the susceptible combinations of single and double crosses, respectively.

The difference between partially resistant and susceptible, and between resistant and partially resistant, combinations in each case was equal on a percentage basis rather than by an absolute amount.[7] The genes multiply the traits of each other instead of combining additively.

[7] SINNOTT, E. W., and DUNN, L. C. PRINCIPLES OF GENETICS. Ed. 3, 408 pp., illus. New York and London. 1939. See p. 134.

COMPLEMENTARY OR MODIFYING FACTORS

In tests for complementary or modifying factors for borer resistance in an inbred line when used in single crosses, the average reaction to borer survival in single-cross combination with several other lines must be known before it can be determined whether or not that line deviates significantly from the average when in combination with some particular inbred line. For this purpose the data plotted in figure 1 were used. It may be noted that only 2 of the 36 single crosses were outside the limits set by the dotted lines. Five percent, or 2 crosses, would be expected to deviate this much from the prediction line through chance alone. Therefore, whatever complementary or modifying action of factors for borer resistance the inbred lines in combinations may have had was not enough to prove important in 1939 when the variability of the data is considered.

Other tests of the possible effect of complementary or modifying factors for borer resistance were provided by data obtained in 1940 and 1941. In 1940 inbred lines Ia. L317, Ill. M14 and 408, Ind. 38–11, Kan. K226 and K230, Mich. MS1, Minn. A340 and A392, Ohio 02, 07, 51A, and 3113, and Wis. CC5, 3922, and 4308 were tested in single-cross combinations on the common parent lines—susceptible A, partially resistant Hy, and resistant R4. In 1941 the following lines were tested in single-cross combination with Hy and R4: Ia. 159, 289, L304A, L317, BL339, and OS420; Ill. A, Pr, 90, and 5120; Ind. P8, WF9, and 38–11; Kan. K226 and K230; Mich. MS1 and 898; Minn. 49, 50, and 374; Ohio 07, 28, 28A, 33, 40B, 51, 51A, 61–67, and 67A; U. S. 4–8, 153, and 187–2; and Wis. CC2, CC4, CC6, CC7, CC8, and CC11. Single crosses made up of susceptible lines were also included in 1941 as standards for comparison.

In 1940 the number of borers in the 2–0–0, 1–1–0, 1–0–1, 0–1–1, and 0–0–2 combinations averaged 3.886, 4.768, 5.850, 7.178, and 8.807 per plant, respectively, on the basis of a geometrical progression with a ratio of 1.227. In 1941 these combinations averaged 1.664, 2.237, 3.006, 4.040, and 5.430 borers per plant, respectively, on the basis of a geometrical progression with a ratio of 1.344. In 1940 crosses A × 51A, A × K226, R4 × K230, R4 × A340, and R4 × CC5 contained significantly fewer borers than the predicted averages of their respective combinations. However, two of these crosses, R4 × K230 and R4 × CC5, were retested in 1941, and they deviated only slightly, one negatively and the other positively, from the averages predicted. Crosses A × 51A, A × K226, and R4 × A340 contained 5.5, 5.1, and 2.8 borers per plant, while the average numbers predicted for these respective combinations were 7.18, 7.18, and 3.89 borers. The differences were not more than 0.6 borer greater than those expected on the basis of the within-strain variability. In 1941 single cross Minn. 49 × R4 was slightly below the lower limit set up on the basis of the within-strain variability as compared with the expected number. It is concluded that whatever complementary or modifying action of factors for borer resistance the inbred lines might have had in combination with lines A, Hy, or R4, in 1940, or with Hy or R4 in 1941, it was not enough to prove important when the variability of the data is considered.

SUMMARY

The average effect of parent inbred lines of dent corn on the survival of larvae of the early summer generation of the European corn borer in single-cross combinations in 1939 was compared quantitatively with their effect in double-cross combinations in 1941 involving, with two exceptions, the same lines used in the single crosses. The lines used had previously been rated as resistant, partially resistant, or susceptible to larval survival. The tests were conducted by infesting each plant by hand with an average of 80 eggs in addition to the natural infestation, and dissecting the plants later to count the mature borers. From a low population of borers in single crosses or double crosses involving resistant lines, the number of borers per plant increased by geometrical progression in the crosses involving successively more susceptible combinations. On the basis of the progressions there were 38.3 percent as many borers in the resistant as in the susceptible combinations of single crosses, as compared with 25.7 percent as many in the resistant as in the susceptible double crosses. A smaller reduction of borers occurred in the presence of a higher infestation in the single crosses than in the double crosses.

A graphical method for determining the possibility of complementary or modifying action of factors for resistance to borer survival is described. One experiment involving 12 inbred lines indicated no effect of complementary or modifying factors in the 36 single crosses tested. From another experiment in which 16 lines were crossed on susceptible line Ill. A, partially resistant Ill. Hy, and resistant Ill. R4 as the common parents, and from still another experiment in which 39 lines were crossed on Hy and R4, it was concluded that whatever complementary or modifying action of factors for borer resistance the inbred lines may have had was not sufficient to be of importance when the variability of the data is considered.

THE SIGNIFICANCE OF AVAILABLE CALCIUM AS A FACTOR LIMITING GROWTH OF AZOTOBACTER AT pH LEVELS BELOW 6.0 [1]

By P. L. GAINEY

Soil bacteriologist, Kansas Agricultural Experiment Station

INTRODUCTION

The following facts concerning the role played by calcium in the physiologic activity of *Azotobacter* have been established: (1) These organisms are usually absent from soils low in calcium and of low pH value, but are present in soils the pH value of which is appreciably above 6.0, a condition associated with a relatively high calcium content (*4, 10*) [2]; (2) liming acid soils adequately to maintain a pH appreciably above 6.0 will transform them into a suitable habitat for *Azotobacter* (*11, 12*); and (3) pure cultures of these organisms will grow abundantly and metabolize atmospheric nitrogen in a modified Ashby medium at pH levels above 6.0, but will not thrive in the same medium buffered to pH values much below 6.0 (*13, 14*). These facts have been interpreted as indicating a maximum H^+ concentration tolerance of approximately 10^{-6} gram mole per liter by these organisms.

More recently Albrecht and associates (*1, 2, 3, 4, 8*) have suggested that the lack of available calcium rather than the high H^+ concentration may be responsible for the failure of leguminous plants and their associated nitrogen-fixing rhizobia to thrive in soils of relatively low pH values. It is of interest to know which of these two concepts is correct.

The known low calcium content of acid soils and the depressing influence of phosphates upon the availability of calcium in artificial media under certain conditions suggested that adequate available calcium for *Azotobacter* activity might not be present in a soil, or in a phosphate-buffered medium, such as Ashby's, if the pH is maintained appreciably below 6.0. McCalla (*15*) has called attention to the presence of only 1.29 mg. equivalents of available calcium per liter in one such medium. Fortunately Albrecht and associates (*5, 6, 7*) have suggested a method whereby it is possible to increase the supply of calcium available to organisms growing in a medium of low pH value, through the use of calcium adsorbed on collodial clay.

The present investigation was undertaken to find out whether the addition of calcium clay to a modified Ashby medium, adjusted to pH levels at which growth of *Azotobacter* otherwise would not take place would render the medium capable of supporting growth of these organisms. McCalla (*15*) has shown that the addition of collodial clay to

Journal of Agricultural Research,
Washington, D. C.

Vol. 76, No. 11
June 1, 1948
Key No. Kans.–102

(265)

such a medium at favorable pH levels markedly increased the rate of nitrogen fixation by *Azotobacter*, but attributed its value to an over-all increase in the availability of nutrient ions in general, rather than to calcium specifically.

<div align="center">PROCEDURE</div>

Two modifications of Ashby's medium were prepared as follows: Na_2HPO_4 and KH_2PO_4 were mixed in ratios to give solutions buffered at pH values of 5.85, 6.00, 6.20, 6.40, and 6.60. To 2.5 gm. of each of these mixtures was added 0.2 gm. NaCl, 0.2 gm. Mg $SO_4.7H_2O$, 0.02 gm. $CaCl_2$, and 0.02 gm. $CaCO_3$.

Medium A was prepared by adding to 1,000 ml. of distilled water, 20.0 gm. of mannitol, 2 drops of 10 percent $FeCl_3$, and 3 mg. of molybdenum as an aqueous solution of MoO_3. To four 125-ml. quantities of this solution (in 250-ml. Erlenmeyer flasks) were added, respectively, 0.368 gm. of the salts mixtured buffered to pH 5.85, 6.00, 6.20, and 6.40. Each of these individual culture solutions contained 1.9 mg. of total calcium.

Medium B was prepared by mixing 150 ml. of a 10-percent suspension of collodial Putnam clay,[3] 250 ml. of saturated $Ca(OH)_2$ (approximately N/25), and water to make 1,000 ml. This was allowed to reach equilibrium, after which 125-ml. quantities for individual cultures were prepared as described for medium A, except that the buffered salts mixtures of 0.2 pH higher values were employed, because it had been observed that the presence of the clay caused a reduction of approximately 0.2 pH in such a medium. Each of these individual culture solutions contained approximately 26.9 mg. of total calcium.

The pH value of each flask of medium was determined with the aid of a Leeds and Northrup glass electrode potentiometer and adjusted if necessary to approximately the desired value, i. e., 5.85, 6.00, 6.20, or 6.40, with NaOH or HCl, after which it was sterilized in an autoclave at 15 pounds pressure. Following sterilization, the pH value was again checked and unless readjustment was necessary these readings were recorded as the initial pH values. These particular pH values were selected because they covered the range within which previous observations had indicated that the maximum tolerance of pure cultures for H^+ concentration might lie, thus providing one or more cultures that would be favorable and one or more unfavorable for growth.

Mannitol-agar slant cultures of *Azotobacter*, grown for 24 hours at 30° C., were washed into a small Erlenmeyer flask containing a few sterile glass beads and thoroughly shaken, in order to secure a homogeneous suspension of cells. Equal quantities of this suspension were pipetted into each flask in a given experiment. A sterile aeration tube passing through a cotton plug was inserted in each flask and connected with the compressed air in a 30° C. incubator. Efforts were made to adjust the flow of air to approximately the same rate in each flask by observing the flow of bubbles. Variations in the flow of air resulting from this procedure appeared to have little effect upon growth as long as bubbling was vigorous.

---

[3] The dialyzed colloidal clay was prepared in the laboratory of Dr. Wm. A. Albrecht, to whom the writer is indebted for supplying adequate quantities for this investigation.

Thirty minutes after aeration was started samples were taken for the initial count. All counts were made with the aid of a Petroff-Hausser counting chamber and are reported in number of cells per cubic millimeter. The pH values were recorded after growth, but these were of questionable value in many instances, because some strains of *Azotobacter* brought about a rapid and marked reduction in the pH value of the medium while others did not.

The presence of an occasional contamination, resulting from the aeration system, could usually be detected by the microscopic appearance of the cells during the counting. However, purity of the cultures was checked by streaking on both nutrient and mannitol agar. No significance was attached to the growth of *Azotobacter* if accompanied by a contaminant. Although the air was passed through several wash bottles before entering the cultures, prolonged aeration resulted in a decrease in the volume; hence incubation was seldom continued longer than 4 days. There is no reason to think the results would have been different after prolonged incubation.

RESULTS

The complete data from each of 3 experiments conducted with the same strain of *Azotobacter* are presented in tables 1, 2, and 3 to indicate the type of quantitative results secured. Fifteen experiments involving 8 different strains are summarized in table 4. In this table the relative amount of growth, as reflected by cell counts, is indicated by + marks.

TABLE 1.—*Growth of Azotobacter (strain 1d, August 5) at different H+ concentrations in the presence and absence of calcium clay*

Medium	Initial state		After incubation of 21 hours	Incubation of 26 hours	
	pH of medium	Cell count	Cell count	pH of medium	Cell count
Modified Ashby (A)	5.80	7,500	6,750	5.80	5,750
Modified Ashby (A)	6.00	7,500	7,750	6.03	6,500
Modified Ashby (A)	6.20	7,500	36,250	6.20	82,500
Modified Ashby (A)	6.38	7,500	237,500	6.40	262,500
Modified Ashby+Ca clay (B)	5.80	7,500	5,750	5.78	6,500
Modified Ashby+Ca clay (B)	6.00	7,500	7,750	6 00	9,000
Modified Ashby+Ca clay (B)	6.20	7,500	38,750	6.20	82,500
Modified Ashby+Ca clay (B)	6.38	7,500	225,000	6.39	187,500

TABLE 2.—*Growth of Azotobacter (strain 1d, September 4) at different H+ concentrations in the presence and absence of calcium clay*

Medium	Initial state		After incubation of 30 hours	After incubation of 46 hours	After incubation of 70 hours	
	pH of medium	Cell count	Cell count	Cell count	pH of medium	Cell count
Modified Ashby (A)	5.82	3,750	3,500	2,500	5.83	1,750
Modified Ashby (A)	5 92	3,750	3,250	2,750	6.00	3,000
Modified Ashby (A)	6.25	3,750	3,750	3,500	6.19	34,250
Modified Ashby (A)	6 46	3,750	32,750	94,500	6.39	(1)
Modified Ashby+Ca clay (B)	5.80	3,750	4,000	3,000	5.87	2,500
Modified Ashby+Ca clay (B)	5.96	3,750	3,500	2,750	6.00	2,500
Modified Ashby+Ca clay (B)	6.28	3,750	5,500	50,000	6.18	(1)
Modified Ashby+Ca clay (B)	6.49	3,750	20,000	44,250	6.38	(1)

[1] After heavy growth, counts were unsatisfactory and were not recorded.

TABLE 3.—*Growth of Azotobacter (Strain 1d, September 18) at different H+ concentrations in the presence and absence of calcium clay*

Medium	Initial state		After incubation of 44 hours	After incubation of 69 hours	After incubation of 93 hours	After incubation of 118 hours	
	pH of medium [1]	Cell count	Cell count	Cell count	Cell count	pH of medium	Cell count
Modified Ashby (A)	5.85	2,250	2,500	2,000	2,750	5 80	2,250
Modified Ashby (A)	6 00	2,250	2,250	2,250	2,000	6 00	1,750
Modified Ashby (A)	6.20	2,250	3,000	142,500	(²)	5 95	(²)
Modified Ashby (A)	6 40	2,250	245,000	(²)	(²)	6 18	(²)
Modified Ashby+Ca clay (B)	5.85	2,250	2,750	3,000	2,750	5.80	3,250
Modified Ashby+Ca clay (B)	6.00	2,250	2,500	2,500	3,000	6 01	4,750
Modified Ashby+Ca clay (B)	6 20	2,250	3,000	2,750	4,000	6 22	51,250
Modified Ashby+Ca clay (B)	6 40	2,250	3,250	4,250	122,500	6.34	(²)

[1] Approximate pH of medium.
[2] After heavy growth, counts were unsatisfactory and were not recorded.

TABLE 4.—*Relative growth [1] of Azotobacter at different H+ concentrations in the presence and absence of calcium clay*

Experiment No	Strain No.	pH range							
		5 70-5 90		5.91-6.10		6 11-6.30		6 31-6.50	
		Medium A	Medium B	Medium A	Medium B	Medium A	Medium B	Medium A	Medium B
1	5	—	—	0	0	?	++	++++	+++
2	A₂	—	—	—	++	?	++++	++++	+++
3	K	—	—	?	?	+++	++	++++	+++
4	d	—	—	0	++	—	0	++++	++++
5	A₂	—	—	—	—	++++	?	++++	++++
6	B₃	—	—	—	—	+	++++	++++	++++
7	A₂	—	—	—	—	++	++	++++	++++
8	K	—	—	—	—	++	++	++++	+++
9	1d	—	—	+++	?	++++	++++	++++	++++
10	M	—	—	?	—	+	?	++++	++++
11	1d	—	—	—	—	+	++++	++++	++++
12	1d	—	—	—	—	++++	++	++++	++++
13	A1	—	—	++	+	+++	+	++++	++++
14	1d	—	—	—	—	++	++	++++	++++
15	1d	—	—	+	++	++++	+++	++++	++++

[1] —No evident growth; ?, questionable growth; +, slight but distinct growth; ++, some growth; +++ good growth; ++++, heavy growth.

It may be recalled that the phosphate salts were mixed to give pH values of 5.85, 6.0, 6.2, and 6.4; however, individual flasks varied somewhat from these values and no special effort was made to adjust them exactly to these points. In table 4 the cultures with various pH values have been grouped under four headings, i. e., 5.70 to 5.90, 5.91 to 6.10, 6.11 to 6.30, and 6.31 to 6.50. In only one instance in this data did the paired cultures of the two media fall in different categories.

DISCUSSION

Rather marked variation was noted in the rate of growth of different strains of *Azotobacter* at comparable pH levels and of the same strain at the same pH value in different experiments, even at the higher and

more favorable pH levels. This variation may have been due, in part at least, to the fact that the time involved in counting cells was so great that only one strain could be grown in a single experiment, and hence identical conditions did not obtain in all experiments. Similar variability in the growth of *Azotobacter* has been observed before.

Differences in the rate of growth might also have resulted in some instances from a retardation of growth coming from a rapid increase in the H^+ concentration produced by some strains and not by others. but this could not account for the differences recorded in tables 1 and 2 since there was practically no change in the H^+ concentrations.

Some differences in the maximum H^+ concentration at which growth took place are evident from a casual study of table 4. In no instance, however, did growth take place below pH 5.9, and with every strain good growth took place at pH values below 6.4. This is the range of pH over which inhibition of growth of *Azotobacter* has been previously reported (*10, 12, 13*).

The interesting point in this connection is that the addition of calcium clay to the modified Ashby medium had little or no influence upon the maximum H^+ concentration tolerated by *Azotobacter*. In experiments 1 and 2 growth occured at a somewhat lower pH level in the presence of the calcium clay, while the reverse was true in experiments 5, 9, and 11. Similarly, somewhat more rapid growth took place in the presence of the clay, near the maximum H^+ tolerated, in experiments 6 and 11; the reverse condition existed in experiments 12 and 13. Since the relative growth of *Azotobacter* in the two media in the 15 experiments recorded in table 4 is indicated by the number of $+$ marks, a comparison of a summation of these for the two media in the various pH categories should give a fair over-all measure of the benefit derived from the presence of calcium clay. Such a comparison indicates that the additional calcium carried by the calcium clay did not facilitate the growth of *Azotobacter* at pH levels where it would not grow otherwise.

SUMMARY

Several strains of *Azotobacter chroococcum* were found to grow abundantly in a modified Ashby medium containing 15 p. p. m. of calcium added as $CaCl_2$ and $CaCO_2$ when the pH was appreciably above 6.0. The same organisms would not grow in this medium if it were buffered at pH levels appreciably below 6.0. The addition of approximately 200 p. p. m. of calcium in the form of calcium clay did not transform this modified Ashby solution into a suitable medium for the growth of *Azotobacter* at pH levels below 6.0. These facts are interpreted as indicating that the lack of available calcium is not the factor responsible for the failure of *Azotobacter* to grow at pH levels below 6.0.

LITERATURE CITED

(1) ALBRECHT, W. A.
    1932. NITROGEN FIXATION AS INFLUENCED BY CALCIUM. 2d Internatl. Soil Sci. Proc. and Papers (1930) 3: 29–39.

(2) ———
    1932. CALCIUM AND HYDROGEN-ION CONCENTRATION IN THE GROWTH AND INOCULATION OF SOYBEANS. Amer. Soc. Agron. Jour. 24: 793–806, illus.

(3) ———
    1933. INOCULATION OF LEGUMES AS RELATED TO SOIL ACIDITY. Amer. Soc. Agron. Jour. 25: 512–522, illus.

(4) ——— and DAVIS, F. L.
    1929. PHYSIOLOGICAL IMPORTANCE OF CALCIUM IN LEGUME INOCULATION. Bot. Gaz. 88: 310–321, illus.

(5) ——— and MCCALLA, T. M.
    1937. ADSORBED CALCIUM ON COLLOIDAL CLAY AND AN ACCESSORY GROWTH FACTOR IN LABORATORY PRODUCTION OF RHIZOBIUM CULTURES. Jour. Bact. 33: 80–81.

(6) ——— and MCCALLA, T. M.
    1938. NITRIFICATION OF AMMONIA ADSORBED ON COLLOIDAL CLAY. Soil Sci. Soc. Amer. Proc. (1937) 2: 263–267, illus.

(7) ——— and MCCALLA, T. M.
    1938. THE COLLOIDAL CLAY FRACTION OF SOIL AS A CULTURAL MEDIUM. Amer. Jour. Bot 25: 403–407, illus.

(8) ——— and POIROT, E. M.
    1930. FRACTIONAL NEUTRALIZATION OF SOIL ACIDITY FOR THE ESTABLISHMENT OF CLOVER. Amer. Soc. Agron. Jour. 22: 649–657, illus

(9) CHRISTENSEN, H. R.
    1915. STUDIEN ÜBER DEN EINFLUSS DER BODENBESCHAFFENHEIT AUF DAS BAKTERIENLEBEN UND DEN STOFFUMSATZ IM ERDBODEN. Centbl. f. Bakt. [etc.] (II) 43: 1–166, illus.

(10) GAINEY, P. L.
    1923. INFLUENCE OF THE ABSOLUTE REACTION OF A SOIL UPON ITS AZOTOBACTER FLORA AND NITROGEN FIXING ABILITY. Jour. Agr. Res. 24: 907–938, illus.

(11) ———
    1925. INOCULATING SOIL WITH AZOTOBACTER. Soil Sci. 20: 73–87, illus.

(12) ———
    1930. A STUDY OF FACTORS INFLUENCING INOCULATION EXPERIMENTS WITH AZOTOBACTER. Kans. Agr. Expt. Sta. Tech. Bul. 26, 66 pp.

(13) ——— and BATCHELOR, H. W.
    1923. INFLUENCE OF THE HYDROGEN-ION CONCENTRATION ON THE GROWTH AND FIXATION OF NITROGEN BY CULTURES OF AZOTOBACTER. Jour. Agr. Res. 24: 759–767.

(14) ——— and FOWLER, E.
    1945. GROWTH CURVES OF AZOTOBACTER AT DIFFERENT PH LEVELS. Jour. Agr. Res. 70: 219–236, illus.

(15) MCCALLA, T. M.
    1937. BEHAVIOR OF LEGUME BACTERIA (RHIZOBIUM IN RELATION TO EXCHANGEABLE CALCIUM AND HYDROGEN ION CONCENTRATION OF THE COLLOIDAL FRACTION OF THE SOIL. Mo. Agr. Expt. Sta. Res. Bul. 256, 44 pp., illus.

(16) ———
    1939. THE ADSORBED IONS OF COLLOIDAL CLAY AS A FACTOR IN NITROGEN FIXATION BY AZOTOBACTER. Soil Sci. 48: 281–286.

EFFECT OF DIFFERENT VARIETIES AND AGES OF SORGHUM ON THE BIOLOGY OF THE CHINCH BUG [1]

By R. G. DAHMS [2]

Associate Entomologist, Division of Cereal and Forage Insect Investigations, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, and associate professor of research in entomology, Oklahoma Agricultural Experiment Station

INTRODUCTION

Measures generally recommended for the control of the chinch bug (*Blissus leucopterus* (Say)) include the construction of barriers to protect susceptible crops, the planting of nonhost crops, and the separation of small grains from corn and sorghums. In the southern part of the infested area in Oklahoma the principal method advocated against this pest, namely, the construction of barriers, is not reliable, because by the time the grains ripen, the insects have usually reached the adult stage and they migrate to corn and sorghums by flight rather than by crawling. A partial solution of the problem of chinch bug control in this area was suggested by the recent development of varieties of plants resistant to insect attack, especially since the many varieties of sorghums growing in the nursery plots at Lawton, Okla., showed different degrees of resistance (*10, 11*).[3] As a step toward determining the cause of this resistance, the biology of the chinch bug has been studied with especial reference to the effect of the sorghum variety on longevity, fecundity, and rate of egg deposition of the adults, and on the mortality and rate of development of the nymphs. The comparative tolerance of certain varieties to injury caused by the feeding of the adults and the host preferences of adults have also been studied. The data presented in this paper are based upon studies made at Lawton during 1936 and 1937.

REVIEW OF LITERATURE

The effect of the host on the biology of an insect has been mentioned by various investigators. McColloch and Salmon (*6*) showed that the

Journal of Agricultural Research,
Washington, D. C.

Vol. 76, No. 12
June 15, 1948
Key No. K-346

(271)

hessian fly (*Phytophaga destructor* (Say)) laid fewer eggs on four varieties of durum wheat than on any of the winter wheats studied. Hodge (*4*) found a wide range of mortality when the grasshopper *Melanoplus differentialis* (Thos.) was reared from egg to adult on different hosts, and Seamans and McMillan (*9*) obtained similar results with the pale western cutworm (*Agrotis orthogonia* Morr.). Wadley (*13*) noted that the mortality of the green bug (*Toxoptera graminum* (Rond.)) was higher on durum wheat than on common wheat. Isely (*5*) reported that the duration of the larval stage and the fecundity of the bollworm (*Heliothis armigera* (Hbn.)) varied considerably when the insect was reared on different host plants. Blanchard and Dudley (*1*) found alfalfa plants on which the pea aphid (*Macrosiphum pisi* (Kltb.)) was unable to maintain a population. Painter (*8*) also found considerable difference in the reproductive ability of the pea aphid on different varieties of alfalfa and even on flowering and vegetative branches of the same plant. According to DeLong and Jones (*3*), the gooseberry aphid (*Kakimia houghtonensis* (Troop)) could not maintain a population on certain plants of the Houghton variety. Winter (*14*) showed that the raspberry aphid (*Amphorophora rubi* (Kltb.)) reproduced much more slowly on some varieties than on others.

Snelling and coworkers (*10*) showed that different varieties of sorghums react differently to heavy infestations of chinch bugs in Oklahoma. Dahms et al. (*2*, *7*) found considerable difference in the rate of development and mortality of chinch bug nymphs as well as in the length of life and number of eggs laid by females when fed different varieties of sorghums and other host plants.

VARIETIES OF SORGHUM USED

The varieties of sorghum chosen for these tests were those representatives of the four major divisions—milos, feteritas, kafirs, and sorgos—that had shown the greatest or the least resistance under field conditions. Since Vinall, Stephens, and Martin (*12*) have published descriptions of most of them, only the most important characters of each variety are given here.

Kansas Orange sorgo × Dwarf Yellow milo (Ks. 24136) is the most resistant selection tested under field conditions. It produces satisfactory yields of grain, but has a buff-colored seed, which is an undesirable characteristic. It is not leafy, has dry, pithy stalks, and is therefore unsatisfactory for forage. This variety has not been distributed to farmers.

Atlas sorgo (C. I. 899) is a cross between Blackhull kafir and Sourless sorgo. It is highly resistant to chinch bugs and well adapted to Oklahoma conditions. This dual-purpose variety is leafy and has white palatable grain and sweet, juicy stalks, which are resistant to lodging.

Kansas Orange (F. C. 9108) is a sorgo that has been grown for many years in eastern Kansas. It shows a great deal of resistance to the chinch bug, but is less desirable than Atlas because of its brown, bitter seed and the tendency of its stalks to lodge.

Blackhull kafir (C. I. 71) is widely grown in Oklahoma, Texas, and Kansas, and has been popular for many years. Its origin is unde-

termined.   Sharon kafir (C. I. 813) is a selection from Blackhull and
is similar to it.   Sharon was used in these tests because it is probably
one of the parents of Wheatland.   Both Blackhull and Sharon kafir
are rather resistant to chinch bug.

Feterita (C. I. 182), an African introduction, formerly was an
important variety of grain sorghum in Texas, Oklahoma, and Kansas.
It is moderately susceptible in its reaction to chinch bugs under field
conditions, but because of its earliness it often escapes serious damage.

Dwarf Yellow milo (C. I. 332) is a popular grain sorghum outside
the chinch bug-infested territory, but is so susceptible to chinch bug
attack that it cannot be grown in sections of the State where chinch
bug is prevalent.

Wheatland (C. I. 918) is a cross between kafir (probably Sharon)
and Dwarf Yellow milo.   It formerly was popular in northwestern
Oklahoma and southwestern Kansas as a grain sorghum suitable for
harvesting with a combine harvester, but, like Dwarf Yellow milo,
it is so susceptible to chinch bugs that it is not grown where they are
abundant.

Honey is a tall, juicy sorgo, which is grown for sirup and silage in
the Southeastern States.   Although not listed in table 1, it is known
to be susceptible to the chinch bug.

COMPARATIVE VARIETAL SUSCEPTIBILITY UNDER FIELD CONDITIONS

The effects of chinch bug infestations on several sorghum varieties
in the field, as reported by Snelling and coworkers (10), are presented
in table 1.   The results for 1931 are not included because the infesta-
tion that year was not heavy enough to show clear-cut varietal differ-
ences.   All these observations were made on plants growing in the
field under natural conditions.

TABLE 1.—*Percentage of plants of several sorghum varieties killed by chinch bugs,*
Lawton, Okla., 1930, 1932, 1933, and 1934 [1]

Variety	Record No.[2]	Percentage of plants killed				
		1930	1932	1933	1934	4-year average
Kansas Orange sorgo × Dwarf Yellow milo.	Ks. 24136	20	2	3	3	7
Altas sorgo	C. I. 899	20	7	20	5	13
Sharon kafir	C. I. 813	42	1	8	5	14
Kansas Orange sorgo	F. C. 9108	38	7	21	2	17
Blackhull kafir	C. I. 71	37	7	37	9	23
Feterita	C. I. 182	98	39	24	43	51
Wheatland	C. I. 918	98	47	100	100	86
Dwarf Yellow milo	C. I. 332	100	100	100	100	100
Average		56 6	26. 3	39. 1	33. 4	38 9

[1] Data taken from Snelling et al. (10).
[2] Ks.—Kansas Agricultural Experiment Station number.   C. I.—Accession number, Division of Cereal Crops and Diseases.   F. C.—Accession number, Division of Forage Crops and Diseases.

The chinch bug infestation was of about the same intensity in 1933
and 1934, being greater in these years than in 1932 but less than in
1930.   Nevertheless, the relative injury among the varieties was com-
parable in the four seasons.   The average mortality due to chinch

bug infestation for the eight varieties ranged from 7 percent on Kansas Orange sorgo × Dwarf Yellow milo (Ks. 24136) to 100 percent on Dwarf Yellow milo. An analysis of the results given in table 1 showed that the differences between years and between varieties were both highly significant.

LABORATORY STUDIES

CONDITIONS AND METHODS

The experiments in 1936 were conducted in a laboratory where the average daily temperatures ranged from 56° to 91° F. The average temperatures during the oviposition period of overwintered, first-generation, and second-generation adults were 74.1°, 83.1°, and 72.2°, respectively, and during development of first-generation, second-generation, and third-generation nymphs they were 76.4°, 84.8°, and 73.3°.

The oviposition and nymph-rearing cage consisted of a transparent celluloid tube about 70 mm. long and 26 mm. in diameter, over one end of which a piece of cloth had been glued. Seedling plants 5 to 6 days old that had been growing in soil out of doors were used as food. Each plant was placed between the halves of a split rubber stopper with the roots extending below and the leaves above. The small end of the stopper was then inserted in a shell vial containing water so that the roots were immersed, while the larger end was fitted tightly into the open end of the celluloid tube so that the leaves were on the inside. The plants were changed daily, at which time egg counts were made. The water in the vials was changed once a week. A pair of chinch bugs was placed in each tube. The male was replaced if it died, but the experiment was discontinued at the death of the female.

EFFECT OF VARIETY ON OVIPOSITION

Oviposition records were kept on nine varieties of sorghum during 1936—the eight listed in table 1 and Honey sorgo. Ten pairs from each of the three generations of bugs were tested on each variety. Adult bugs of the overwintered generation were collected from bunch grass just before they were ready to leave their hibernating quarters. The bugs of the later generations were collected in the field when in the fifth instar, from sorghum of the same varieties on which they were to be placed in the laboratory.

The results of oviposition are given in table 2. Dwarf Yellow milo was the only variety on which all the females of the overwintered generation laid eggs. Those feeding on Atlas sorgo, feterita, and Kansas Orange sorgo laid an average of less than 1 egg per female. All 10 females of the first-generation feeding on Dwarf Yellow milo, Wheatland, and Honey sorgo laid eggs, but none of those feeding on Kansas Orange sorgo oviposited. Although 9 of the 10 females that fed on Atlas sorgo oviposited, the average number of eggs per female was only 8.9 as compared with 132.9 on Dwarf Yellow milo and 139.7 on Wheatland. On most varieties fewer eggs were laid by second-generation than by first-generation bugs. The average number of eggs laid per female for second-generation bugs ranged from 0.7 on Kansas Orange sorgo to 56.9 on Wheatland.

TABLE 2.—*Oviposition of 3 generations of chinch bugs on 9 varieties of sorghum in laboratory studies* [1]

Variety	Overwintered generation			First generation				Second generation				Total			
	Females laying eggs	Eggs laid per female		Average temperature	Females laying eggs	Eggs laid per female		Average temperature	Females laying eggs	Eggs laid per female		Average temperature	Females laying eggs	Eggs laid per female	
		Range	Average			Range	Average			Range	Average			Range	Average
	Number	*Number*	*Number*	*°F.*	*Number*	*Number*	*Number*	*°F.*	*Number*	*Number*	*Number*	*°F.*	*Number*	*Number*	*Number*
Dwarf Yellow milo	10	44–228	123.0	72.4	10	9–211	132.9	82.9	10	6–135	42.3	73.1	30	6–228	99.4
Wheatland	8	0–192	82.5	71.3	10	39–278	139.7	82.4	10	7–119	56.9	71.8	28	0–278	93.0
Honey sorgo	9	0–144	72.8	74.1	10	5–95	43.4	83.4	9	0–60	21.7	82.0	28	0–144	46.0
Blackhull kafir	9	0–25	10.8	71.9	9	0–48	18.2	83.1	7	0–109	34.7	71.8	25	0–109	21.2
Sharon kafir	4	0–101	23.8	72.4	7	0–20	7.5	82.0	6	0–72	17.7	76.4	17	0–101	16.3
Kansas Orange sorgo × Dwarf Yellow milo	2	0–26	4.9	71.4	5	0–32	7.5	80.9	6	0–49	9.4	78.7	13	0–49	7.3
Atlas sorgo	2	0–6	.8	67.1	9	0–22	8.9	82.0	1	0–21	2.1	85.1	12	0–22	3.9
Feterita	1	0–1	.1	68.2	5	0–16	4.2	81.7	2	0–7	.8	85.0	8	0–16	1.7
Kansas Orange sorgo	1	0–1	.1	67.3	0	0	0	81.7	3	0–3	.7	85.0	4	0–3	1.3
Average			35.4				40.3				20.7				32.1
Difference required for significance at odds of 19 to 1															40

[1] 10 pairs of bugs were used with each host plant for each generation.

When the results for all three generations are considered, bugs feeding on Dwarf Yellow milo and Wheatland produced on an average significantly more eggs than any of the bugs feeding on the other varieties. The differences between the average number of eggs per generation were not statistically significant.

EFFECT OF VARIETY ON LONGEVITY

The longevity of the females feeding on the nine varieties for the three generations is shown in table 3. Bugs of the overwintered gen-

TABLE 3.—*Longevity of 3 generations of chinch bug females on 9 varieties of sorghum in laboratory studies*

Variety	Overwintered generation			First generation			Second generation			Total longevity	
	Average temperature	Longevity		Average temperature	Longevity		Average temperature	Longevity		Range	Average
		Range	Average		Range	Average		Range	Average		
	°F.	Days	Days	°F.	Days	Days	°F.	Days	Days	Days	Days
Wheatland	71.3	35–103	74.8	82.4	32–83	64.0	71.8	15–157	59.0	15–157	65.9
Dwarf Yellow milo	72.4	35–115	88.1	82.9	12–83	64.2	73.1	15–120	36.0	12–120	56.8
Honey sorgo	74.1	47–134	95.2	83.4	18–77	40.4	82.0	4–59	27.2	4–134	54.3
Blackhull kafir	71.9	33–111	61.0	83.1	4–83	34.3	71.8	4–157	49.4	4–157	48.2
Sharon kafir	72.4	28–115	63.9	82.0	20–51	30.8	76.4	6–87	28.0	6–115	40.9
Kansas Orange sorgo × Dwarf Yellow milo	71.4	28 105	50.8	80 9	9–34	18.8	78.7	4–73	28.0	4–105	32.5
Feterita	68.2	28–71	41.1	81.7	6–43	27.9	85.0	4–26	12.8	4–71	27.3
Atlas sorgo	67.1	28–45	35.0	82.0	4–43	24.0	85.1	5–31	18.8	4–45	25 9
Kansas Orange sorgo	67.3	28–56	35.6	81.7	6–39	15 1	85.0	4–26	13.6	4–56	21.4
Average			60.6			33.5			30.3		41.5
Difference required for significance at odds of 19 to 1											18.2

eration lived significantly longer than those of the other two generations. It should be kept in mind that the data for the longevity of overwintered bugs represents only their average longevity after they were brought into the laboratory. In most cases several days elapsed before they began laying eggs.

The average longevity of bugs of all three generations was greatest on Wheatland. Tnen in order came Dwarf Yellow milo, Honey sorgo, Blackhull kafir, Sharon kafir, Kansas Orange sorgo × Dwarf Yellow milo, feterita, Atlas sorgo, and Kansas Orange sorgo. There is a very close correlation between the number of eggs laid and the longevity of the female.

EFFECT OF VARIETY ON SIZE OF ADULTS

The relation between the size of adult chinch bugs and the variety of sorghum that they fed upon during their nymphal stage is shown in table 4. The bugs reared on the susceptible varieties, Wheatland and Dwarf Yellow milo, were the largest, and those reared on the resistant Kansas Orange were the smallest. Statistically, the differences in body length between generations were barely significant, but the differences in body length of bugs reared on different varieties were highly significant.

TABLE 4.—*Average body length of chinch bugs reared on different varieties of sorghum in laboratory studies*

Variety	Average body length of adults			
	First generation	Second generation	Third generation	Average
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*
Wheatland	3.70	3.78	3.85	3.78
Dwarf Yellow milo	3.80	3.76	3.61	3.72
Honey sorgo	3.72	3.74	3.41	3.62
Sharon kafir	3 72	2.71	3.40	3.61
Blackhull kafir	3.61	3.54	3.55	3 57
Kansas Orange sorgo × Dwarf Yellow milo	3 48	3.49	3.50	3 49
Atlas sorgo	3.60	3.40	3.36	3.45
Feterita	3 34	3.57	3.07	3.35
Kansas Orange sorgo	3 06	3.35	(1)	3.20
Average	3.56	3.59	3.47	3 54
Differences required for significance at odds of 19 to 1				21

[1] All dead before adult stage reached. Average of first and second generations used for analysis of variance.

EFFECT OF VARIETY ON RATE OF NYMPHAL DEVELOPMENT

A total of 307 chinch bugs was reared from egg to adult on 9 varieties of sorghum. Bugs were brought into the laboratory from the field and placed on the same varieties as those from which they had been collected. The eggs laid by females feeding on each variety were kept separate, and the resulting nymphs were placed on the same variety as that upon which the female had been feeding when the eggs were laid.

The records for nymphal development are summarized in table 5. The nymphal period ranged from 18 to 67 days, depending on the

TABLE 5.—*Days required for development of 3 generations of chinch bug nymphs reared in the laboratory on 9 varieties of sorghum*

Variety	Days from hatching to adult			
	First generation	Second generation	Third generation	Average
Dwarf Yellow milo	37.4	27.0	30.1	31.50
Wheatland	41.0	25.2	28.4	31.53
Honey sorgo	43.3	27.0	29.5	33.27
Blackhull kafir	39.4	27.9	34.4	33.90
Kansas Orange sorgo × Dwarf Yellow milo	39.1	27.2	43.9	36.73
Kansas Orange sorgo	43.0	31.1		37.05
Atlas sorgo	42.8	32 8	38.0	37.87
Sharon kafir	41.5	28.2	44.0	37.90
Feterita	46 5	29.3	49.0	41.60
Average	41.6	28.4	37.2	35.7

variety of sorghum and the temperature. In most cases nymphs feeding on those varieties that are most susceptible under field conditions matured faster than nymphs feeding on resistant varieties. On feterita, however, which is a moderately susceptible variety under field conditions, development was slower than on any of the resistant varieties. The fastest development recorded for any of the first-generation nymphs was 31 days for one nymph on Honey sorgo. The longest time required for nymphal development was 51 days,

as recorded for three nymphs, one each on Sharon kafir, feterita, and Honey sorgo.

The development of second-generation nymphs was slowest on Atlas sorgo. Second-generation nymphs developed faster on all varieties than those of the first generation because of the higher temperature, the period from egg to adult ranging from 20 to 42 days.

Third-generation nymphs showed the widest variation in rate of development. One nymph feeding on Wheatland developed from egg to adult in 18 days, and two nymphs, one on Sharon kafir and one on Kansas Orange sorgo × Dwarf Yellow milo, required 67 days to complete their development. There was a difference of 20.6 days in the average time required for nymphs to mature on Wheatland and feterita. This extreme variability was due to the warm weather prevailing during the first 28 days of this experiment, followed by a sudden change to cool weather, which lasted throughout the fall. Thus, the nymphal period of those bugs that had not reached the adult stage by the end of the twenty-eighth day of the experiment was greatly lengthened.

There was some variation in the length of stadia of nymphs feeding on each of the different varieties, but as a general rule this difference was not great, and in all cases the first stadium was the longest. Little difference existed between the second, third, and fourth stadia, but the fifth stadium was longer.

When the data were analyzed it was found that the differences between generations in rate of development were highly significant. Considering all the data, the differences in rate of development on the different varieties were not significant. Nevertheless, when the three most susceptible varieties were compared with the three most resistant varieties by Student's method, the odds were about 255 to 1 against a difference as great as this being due to chance alone.

EFFECT OF VARIETY ON MORTALITY OF NYMPHS

The nymphs were reared in mass culture until they were 5 days old, when they were put into individual cages. The mortality of nymphs was very high the first few days after hatching, but no records were kept while they were in mass culture, because it was difficult to distinguish between mortality due to handling and that due to variety of host.

The mortality of nymphs for all three generations is given in table 6. The lowest for first-generation nymphs was on Dwarf Yellow milo and the highest on Kansas Orange sorgo. Blackhull kafir produced the lowest mortality in the second generation, and Atlas sorgo the highest. The relative mortality of third-generation nymphs was similar to that of the first generation. However, the mortality of first instars was much greater than in the first and second generations.

The average nymphal mortality for all three generations was lowest for nymphs reared on Dwarf Yellow milo. Then in order came Wheatland, Blackhull kafir, Kansas Orange sorgo × Dwarf Yellow milo, Honey sorgo, Sharon kafir, feterita, Atlas sorgo, and Kansas Orange sorgo. From the analysis of variance it was found that the differences in the average mortality between generations and between varieties were both highly significant.

TABLE 6.—*Percent mortality of 3 generations of chinch bug nymphs reared in the laboratory on 9 varieties of sorghum*

Variety	Percent mortality of—			
	First generation	Second generation	Third generation	Average
Kansas Orange sorgo	92	65	100	85. 7
Atlas sorgo	60	80	96	78. 7
Feterita	56	65	80	67. 0
Sharon kafir	48	40	76	54. 7
Honey sorgo	36	45	52	44. 3
Kansas Orange sorgo × Dwarf Yellow milo	24	45	40	36. 3
Blackhull kafir	20	30	52	34. 0
Wheatland	12	35	48	31. 7
Dwarf Yellow milo	12	45	28	28. 3
Average	40. 0	50. 0	63. 6	51. 2
Difference required for significance at odds of 19 to 1				8. 9

HOST PREFERENCE

Laboratory tests were conducted in 1936 to study the preference of the chinch bug for different varieties of sorghum. A wooden cage 22 inches long, 6 inches wide, and 6 inches high, equipped with a sliding glass top, was constructed. In each end of the cage were large holes covered with fine-mesh copper screen, for ventilation. The cage was supported on 4 metal legs 10 inches long which were clamped on each corner. In the bottom of the cage 10 holes were bored 2 inches apart. One-ounce medicine bottles with screw tops were inserted in these holes from underneath the cage and filled with water. Seedling plants of two varieties to be compared were placed alternately in these bottles, so that the roots were submerged in the water and the upper portions extended into the cage. Cotton was packed around each plant at the mouth of the bottle to prevent escape of the insects. Adult chinch bugs were then collected from the field and placed in the cage. At intervals of about 2 hours the insects on each plant were counted and then removed and distributed evenly in the cage.

Atlas sorgo was compared successively with the eight other varieties in these tests. Table 7 shows the number and percentage of bugs attracted to each variety in five trials. These insects showed a distinct preference for Dwarf Yellow milo, Wheatland, and Honey sorgo, over Atlas sorgo, but only a very slight preference for the other varieties over Atlas sorgo.

HOST TOLERANCE

To study the tolerance of different varieties of sorghum to a uniform infestation of chinch bugs, single plants grown in 6-inch flowerpots (fig. 1), three of each variety, were brought into the laboratory when about 10 inches high and covered with 5- by 8-inch celluloid tubes. One hundred adult chinch bugs were placed on each plant.

Tolerance studies were made with eight varieties of sorghum (table 8). An average of only 88.3 hours was required for the bugs to kill Honey sorgo and Dwarf Yellow milo plants as compared with 131.7 hours for Atlas sorgo. Two plants of Atlas sorgo lived 135 hours, whereas all the plants of Honey sorgo and Dwarf Yellow milo were dead at the end of 95 hours. When these data were tested by analysis of variance, the differences between varieties were found to be highly significant.

790476—48——2

TABLE 7.—*Preference of chinch bugs for different varieties of sorghum as compared with Atlas sorgo, in laboratory tests*

Variety	Bugs feeding on plants							Bugs on each variety
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Total	Average per trial	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Dwarf Yellow milo	115	95	96	34	43	383	76.6	64.26
Atlas sorgo	68	44	36	31	34	213	42.6	35.74
Blackhull kafir	91	92	84	42	40	349	69.8	52.32
Atlas sorgo	79	94	82	41	22	318	63.6	47.68
Honey sorgo	123	100	87	61	50	421	84.2	62.83
Atlas sorgo	68	66	50	36	29	249	49.8	37.17
Kansas Orange sorgo	42	61	149	46	45	343	68.6	49.86
Atlas sorgo	50	61	159	40	35	345	69.0	50.14
Wheatland	140	141	129	109	98	617	123.4	60.14
Atlas sorgo	116	103	77	52	61	409	81.8	39.86
Kansas Orange sorgo X Dwarf Yellow milo	118	85	71	75	74	423	84.6	53.61
Atlas sorgo	117	72	56	58	63	366	73.2	46.39
Sharon kafir	119	120	114	108	140	601	120.2	50.42
Atlas sorgo	133	103	108	99	148	591	118.2	49.58
Feterita	66	130	140	74	68	478	95.6	50.47
Atlas sorgo	60	150	135	59	65	469	93.8	49.53

FIGURE 1.—Cages used for tolerance studies with the chinch bug.

TABLE 8.—*Tolerance of 8 varieties of sorghum to a uniform infestation of chinch bugs under controlled conditions in the laboratory*

Variety	Condition of plant after indicated number of hours [1]											Average hours of life of plant
	35	45	55	65	75	85	95	105	115	125	135	
Honey sorgo	A	B	C	E	E	F						88.3
	A	A	B	C	C	E	F					
	A	A	B	D	E	F						
Dwarf Yellow milo	A	A	B	B	B	E	F					88.3
	A	B	B	B	C	F						
	A	A	B	C	D	F						
Wheatland	A	A	B	C	C	D	F					98.3
	A	A	A	B	C	C	E	F				
	A	A	B	C	D	E	F					
Kansas Orange × Dwarf Yellow milo	A	A	A	B	C	D	E	F				101.7
	A	A	B	B	D	E	F					
	A	A	A	B	C	C	E	F				
Blackhull kafir	A	B	B	C	C	D	E	E	F			108.3
	A	A	A	B	C	D	E	E	F			
	A	B	C	C	D	E	F					
Feterita	A	B	B	C	C	D	D	D	E	F		121.7
	A	A	A	B	C	D	D	D	E	F		
	A	B	B	B	C	D	E	E	F			
Kansas Orange sorgo	A	A	A	A	B	D	D	E	E	F		128.3
	A	A	A	A	B	D	D	E	E	F		
	A	A	A	A	B	C	D	D	E	E	F	
Atlas sorgo	A	A	A	A	B	B	C	C	D	F		131.7
	A	A	A	A	B	B	B	C	C	E	F	
	A	A	A	A	B	B	C	C	D	E	F	

[1] A, no injury; B, basal leaves slightly wilted; C, part of basal leaves permanently wilted; D, about half of the leaves of the plant permanently wilted; E, entire plant nearly dead; F, entire plant dead.

FIELD STUDIES

CONDITIONS AND METHODS

In 1937 the effect of different varieties of older sorghum plants on the biology of the chinch bug was studied under field conditions on Dwarf Yellow milo, feterita, Blackhull kafir, and Atlas sorgo. Three series of each variety were used—(1) plants 20 to 30 days old (average 25 days), (2) plants 40 to 50 days old (average 45 days), and (3) plants at the heading stage.

The bugs were confined in cages on plants growing in the field. These cages consisted of celluloid tubes about 7 by 2.5 cm. (fig. 2), with a cork stopper covered with muslin in each end. An ellipsoid hole about the middle of the side of the tube was also covered with muslin, which was attached with waterproof glue. Each cage was fastened to a sorghum stalk with a fabric-covered elastic band so that the hole was next to the stalk. Bugs placed in these cages appeared to have no difficulty in feeding through the cloth. The cages were placed on the north side of the plants and as much in the shade as possible. Under these conditions the temperature in the cages ordinarily ranged from 2° to 6° F. higher than outside shade-air temperatures. The shade-air temperatures during these experiments are shown in figure 3.

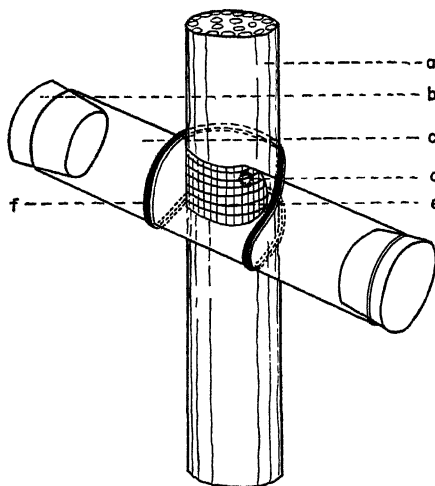FIGURE 2.—Cage used to confine chinch bugs on plants growing in the field: *a*, Sorghum plant; *b*, cork stopper; *c*, celluloid cage; *d*, chinch bug; *e*, feeding area; *f*, elastic band.

The bugs used for oviposition and longevity studies were collected in the field while in the fifth instar. The first-generation bugs were
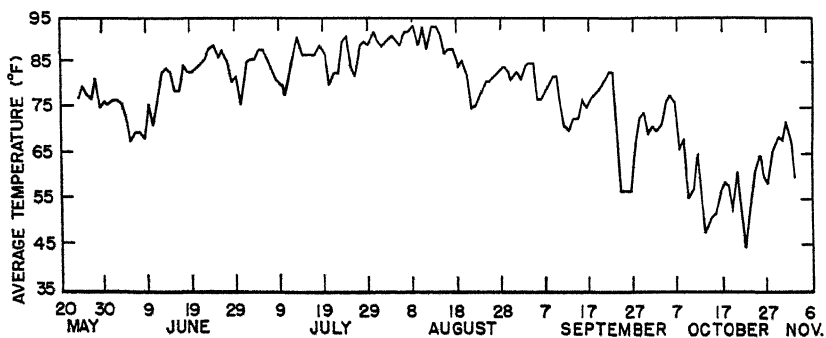

FIGURE 3,—Average daily shade-air temperatures in sorghum fields at Lawton, Okla., May to November 1937.

collected from spring barley and the second-generation from the same varieties of sorghum of which they were to be confined in the cages. These fifth instars were then placed on plants of the same variety and age as those on which they were later to be tested. When the bugs reached the adult stage, one pair was placed in each cage and one cage attached to each plant. The nymphs used for studying the rate of development were from eggs laid by bugs that had fed on plants of the same variety and age as those upon which they were later to be tested.

EFFECT OF VARIETY AND AGE OF PLANT ON OVIPOSITION

The oviposition records of chinch bugs on four varieties of sorghum at three stages of growth are given in table 9.

The bugs feeding on Dwarf Yellow milo laid the most eggs, but the differences in oviposition on varieties were not so great as when similar tests were made with seedling plants in the laboratory. Bugs feeding on Dwarf Yellow milo laid nearly twice as many eggs as those feeding on Atlas sorgo or Blackhull kafir. Feterita was intermediate between Dwarf Yellow milo and Blackhull kafir in this respect.

TABLE 9.—*Number of eggs per female of 2 generations of chinch bugs on 4 varieties of sorghum at 3 different ages growing in the field*

Variety	Generation of bugs	Eggs laid per female on—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Number	*Number*	*Number*	*Number*
Dwarf Yellow milo	First	123.9	201.5	203.1	
	Second	113.4	219.9	270.8	188 8
Feterita	First	88.4	135.8	167.4	
	Second	86 2	230.5	183.9	148.7
Blackhull kafir	First	61.9	145 3	107.7	
	Second	72 9	116.5	123.6	104 6
Atlas sorgo	First	78.9	98 0	101.2	
	Second	32.8	158.8	118.0	97.9
Average		82 3	163.3	159 5	135 0

Fewer eggs were laid on 25-day-old plants of all varieties than on 45-day-old or headed plants. Bugs feeding on Dwarf Yellow milo laid more eggs on headed plants than on plants of either of the other two stages, whereas bugs feeding on feterita, Blackhull kafir, and Atlas sorgo laid the most eggs on 45-day-old plants. The analysis of variance showed that the differences in numbers of eggs laid on different varieties and on plants of different ages are highly significant, but that the difference between generations is not significant.

EFFECT OF VARIETY AND AGE OF PLANT ON LONGEVITY

Longevity records of the females used in the oviposition experiment (table 10) showed no correlation between the length of life and the number of eggs laid (table 9). Bugs feeding on feterita lived on an average 10 days longer than bugs feeding on the other varieties.

TABLE 10.—*Longevity of 2 generations of chinch bugs on 4 varieties of sorghum at 3 different ages in the field*

Variety	Generation of bugs	Longevity of bugs on—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Days	*Days*	*Days*	*Days*
Dwarf Yellow milo	{First	31 6	47 2	43.6	
	{Second	36 2	46.7	54.2	43.2
Feterita	{First	43 2	46.3	53.3	
	{Second	41.1	80.2	56.7	53.5
Blackhull kafir	{First	29.0	53.7	33.1	
	{Second	41.8	48.6	55.5	43.6
Atlas sorgo	{First	41.5	43.6	42 5	
	{Second	22.1	54 4	50.2	42.4
Average		35.8	52.6	48.6	45.7

The age of the plant, however, had a similar effect on the longevity as on oviposition of the bugs, for they lived a shorter time on 25-day-old plants than on plants of either of the other two age groups. Bugs feeding on Dwarf Yellow milo lived longest on headed plants, while those feeding on feterita, Blackhull kafir, and Atlas sorgo plants lived longest on 45-day-old plants. When these data were tested by analysis of variance, the differences in longevity among varieties and between generations were not found to be significant, but the differences between the three ages of plants were highly significant.

EFFECT OF VARIETY AND AGE OF PLANT ON SIZE OF ADULTS

The average body lengths of three generations of chinch bugs reared on the four varieties are shown in table 11. Bugs reared on

TABLE 11.—*Average body length of adult chinch bugs reared in the field on 4 varieties of sorghum at 3 different ages*

Variety	Average body length of adults on—			
	25-day-old plants	45-day-old plants	Headed plants	Average of all plants
	Millimeters	*Millimeters*	*Millimeters*	*Millimeters*
Dwarf Yellow milo	3.84	3.80	3 68	3.77
Feterita	3.68	3.80	3 63	3.70
Blackhull kafir	3.63	3.76	3.76	3.72
Atlas sorgo	3.67	3.60	3.42	3.5

Dwarf Yellow milo were, on an average, distinctly larger than bugs reared on Atlas sorgo but only slightly larger than those reared on feterita or Blackhull kafir. In the analysis of variance, none of these differences were found to be significant. The age of the plants did not significantly affect the size of the bugs.

EFFECT OF VARIETY AND AGE OF PLANT ON RATE OF NYMPHAL DEVELOPMENT

When nymphs were reared from egg to adult on four varieties of sorghum under field conditions, they developed most rapidly on Dwarf Yellow milo (table 12). The rate of development on feterita, Black-

TABLE 12.—*Days required for development of 3 generations of chinch bug nymphs in the field on 4 varieties of sorghum at 3 different ages*

Variety	Generation of bugs	Time required for development—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Days	*Days*	*Days*	*Days*
Dwarf Yellow milo	First	35.7	32.5	30.5	
	Second	22 9	24.5	24.6	36 1
	Third	46 7	50.5	56.6	
Feterita	First	34 3	35.0	36 5	
	Second	26.7	27.5	27.0	40.4
	Third	60.3	58.9	57.3	
Blackhull kafir	First	33.5	39.0	43.8	
	Second	28.2	25.6	27.0	41.3
	Third	59.3	54.9	60.7	
Atlas sorgo	First	39 0	38.0	38 0	
	Second	26.8	25.5	27.0	39.4
	Third	54.2	51.3	55 0	
Average		39 0	38.6	40 3	39.3

hull kafir, and Atlas sorgo was about the same. Second-generation bugs developed much faster than first- and third-generation bugs, owing to higher prevailing temperatures. Analysis of variance showed that the differences in the rate of development on the different varieties and in different generations were highly significant, but on the plants of different ages they were not significant.

EFFECT OF VARIETY AND AGE OF PLANT ON MORTALITY OF NYMPHS

The mortality of nymphs feeding on the four varieties is shown in table 13. The average mortality for each generation was lowest on Dwarf Yellow milo, and in all except 25-day-old plants the mortality was highest on Atlas sorgo. Nymphs feeding on Blackhull kafir had a higher mortality than those feeding on feterita. Analysis of variance showed that the differences in mortality between varieties were significant. The average mortality on all four varieties was slightly higher on headed plants than on the younger plants, but the difference was not significant. The differences in mean mortalities between the generations were significant.

TABLE 13.—*Mortality of 3 generations of chinch bug nymphs on 4 varieties of sorghum at 3 different ages growing in the field*

Variety	Generation of bugs	Mortality of nymphs on—			
		25-day-old plants	45-day-old plants	Headed plants	Average of all plants
		Percent	*Percent*	*Percent*	*Percent*
Dwarf Yellow milo	First	72.0	84.0	84.0	
	Second	68.0	60.0	52.0	62.7
	Third	36.0	48.0	60 0	
Feterita	First	88.0	96.0	92.0	
	Second	76.0	68.0	64.0	74.7
	Third	76.0	36.0	76.0	
Blackhull kafir	First	92.0	88.0	100.0	
	Second	80.0	68.0	68.0	77.8
	Third	68.0	64.0	72.0	
Atlas sorgo	First	76.0	96.0	96.0	
	Second	80.0	84.0	84.0	83.1
	Third	80.0	76.0	76.0	
Average		74.3	72.3	68.7	71.8

DISCUSSION

Little is known as to the basis of chinch bug resistance in plants, but it is believed to be related to the condition or composition of the cell sap rather than to any readily observable morphological characters of the plant. The laboratory data on the length of life, fecundity, rate of development, and size of the insect indicate that seedlings of different sorghum varieties do not serve equally well as food. Generally chinch bugs feeding on those varieties that are susceptible under field conditions live longer and lay more eggs than those feeding on resistant varieties. The nymphs also develop into larger adults. This is not true in all cases, however, feterita being the outstanding exception. Kansas Orange sorgo X Dwarf Yellow milo showed transgressive segregation according to field results but not in the laboratory tests.

Whether fecundity, mortality, and size are all influenced by one genetic factor, each by a separate factor, or each by several factors, is not known. The fact that a low fecundity was accompanied by a high death rate would indicate that all may be affected by the same factor, or by a group of factors that are closely linked. Whether these differences are due to absence, presence, or to the quantity of certain food materials in the cell sap is not known.

The differences between chinch bugs reared on different varieties of sorghum were not so marked for older plants under field conditions as for seedling plants tested in the laboratory. In fact, under field conditions the only significant differences due to variety were in number of eggs laid, nymphal mortality, and rate of nymphal development. The apparent discrepancy respecting the relative position of resistance of feterita has been cleared up to some extent by field-cage studies. In the laboratory this variety, which is moderately susceptible under field conditions, has always reacted as resistant when the effect of host on the biology of the chinch bug was used as a criterion. In studies with older plants bugs laid more eggs and the mortality of nymphs was lower on this variety than on either Blackhull kafir or Atlas sorgo. The reactions of the bugs as determined by both field-cage tests and field observations indicate it to be more susceptible than Blackhull kafir or Atlas sorgo.

SUMMARY

Varieties of sorghum differ greatly in their ability to withstand the attack of the chinch bug (*Blissus leucopterus* (Say)). In general, under field conditions the milos are very susceptible, the feteritas susceptible, and the kafirs and sorgos rather resistant.

A study of the effect of seedling plants of nine varieties of sorghum on the biology of the chinch bug showed that chinch bug females feeding on Dwarf Yellow milo laid more eggs than those feeding on any of the other varieties. In the order of their increasingly detrimental effect on chinch bug fecundity, came Wheatland, Honey sorgo, Blackhull kafir, Sharon kafir, Kansas Orange sorgo X Dwarf Yellow milo, Atlas sorgo, feterita, and Kansas Orange sorgo.

There was a marked difference in the longevity of females when confined with different varieties of sorghum, but longevity was not

always correlated with the total number of eggs laid. Chinch bug females lived longest on Wheatland, and the shortest time on Kansas Orange sorgo.

Nymphs reared on the susceptible varieties were larger than those reared on the resistant varieties. There were some differences in the rate of development of nymphs on the several varieties. While these differences among all varieties were not significant when tested by analysis of variance, comparison by Student's method of results for the three most resistant and the three most susceptible varieties showed that nymphs develop significantly faster on the susceptible varieties. Mortality of nymphs was greatest on Kansas Orange sorgo and least on Dwarf Yellow milo.

Under laboratory conditions adult chinch bugs showed a preference for susceptible varieties over resistant varieties. The resistant varieties showed the greatest tolerance to a uniform infestation of adult chinch bugs.

The effect of four varieties of sorghum at three stages of growth on the biology of the chinch bug was also studied under field conditions. The bugs laid the largest number of eggs on Dwarf Yellow milo, the next largest on feterita, and the smallest number on Blackhull kafir and Atlas sorgo. Fewer eggs were laid on all varieties of 25-day-old plants than on plants 45 days old or on plants in the heading stage. Chinch bugs lived longer on 45-day-old and headed plants than on 25-day-old plants. There was no apparent correlation between resistance of plants and longevity of adult chinch bugs.

When chinch bugs were reared in field cages, the adults were distinctly larger on Dwarf Yellow milo than on Atlas sorgo, those reared on feterita or Blackhull kafir being intermediate in size. Generally, bugs reared on headed plants were smaller than those reared on younger plants. Under field conditions nymphs developed faster on Dwarf Yellow milo than on the other three varieties. The age of plant did not affect the rate of development of nymphs. Nymph mortality was lowest on Dwarf Yellow milo, highest on Atlas sorgo, and intermediate on feterita and Blackhull kafir.

It is believed that the condition or composition of the cell sap rather than any readily observable morphological characters of the plant is responsible for the differences observed on the biology of the chinch bug when feeding on different varieties and ages of sorghum. Whether these differences are due to absence, presence, or quantities of certain food materials in the cell sap is not known.

LITERATURE CITED

(1) BLANCHARD, R. A., and DUDLEY, J. E., JR.
    1934. ALFALFA PLANTS RESISTANT TO THE PEA APHID. Jour. Econ. Ent 27: 262–264.
(2) DAHMS, R. G., SNELLING, R. O., and FENTON, F. A.
    1936. EFFECT OF SEVERAL VARIETIES OF SORGHUM AND OTHER HOST PLANTS ON BIOLOGY OF THE CHINCH BUG. Jour. Econ. Ent. 29: 1147–1153, illus.
(3) DELONG, D. M., and JONES, M. P.
    1926. CONTROL MEASURES FOR THE HOUGHTON GOOSEBERRY APHIS WITH SPECIAL REFERENCE TO PLANT RESISTANCE. Jour. Econ. Ent. 19: 40–43, illus.

(4) HODGE, CHARLES, IV.
    1933. GROWTH AND NUTRITION OF MELANOPLUS DIFFERENTIALIS THOMAS
        (ORTHOPTERA: ACRIDIDAE) I. GROWTH ON A SATISFACTORY
        MIXED DIET AND ON DIETS OF SINGLE FOOD PLANTS. Physiol.
        Zool. 6: 306–328, illus.
(5) ISELY, D.
    1935. RELATION OF HOSTS TO ABUNDANCE OF COTTON BOLLWORM. Ark.
        Agr. Expt. Sta. Bul. 320, 30 pp., illus.
(6) McCOLLOCH, J. W., and SALMON, S. C.
    1918. RELATION OF KINDS AND VARIETIES OF GRAIN TO HESSIAN-FLY
        INJURY. Jour. Agr. Res. 12: 519–527.
(7) OKLAHOMA AGRICULTURAL EXPERIMENT STATION.
    1936. CHINCH-BUG CONTROL STUDIED FROM SEVERAL ANGLES. In
        Science Serving Agriculture. Okla. Agr. Expt. Sta. [Bien.] Rpt.
        1934–36, pp. 34–39, illus.
(8) PAINTER, R. H.
    1936. THE FOOD OF INSECTS AND ITS RELATION TO RESISTANCE OF PLANTS
        TO INSECT ATTACK. Amer. Nat. 70: 547–566.
(9) SEAMANS, H. L., and McMILLAN, E. L.
    1935. THE EFFECT OF FOOD PLANTS ON THE DEVELOPMENT OF THE PALE
        WESTERN CUTWORM (AGROTIS ORTHOGONIA MORR.). Jour. Econ.
        Ent. 28:421–425.
(10) SNELLING, R. O., PAINTER, R. H., PARKER, J. H., and OSBORN, W. M.
    1937. RESISTANCE OF SORGHUMS TO THE CHINCH BUG. U. S. Dept. Agr.
        Tech. Bul. 585: 56 pp., illus.
(11) SNELLING, R. O., and DAHMS, R. G.
    1937. RESISTANT VARIETIES OF SORGHUM AND CORN IN RELATION TO
        CHINCH BUG CONTROL IN OKLAHOMA. Okla. Agr. Expt. Sta. Bul.
        232, 22 pp., illus.
(12) VINALL, H. N., STEPHENS, J. C., and MARTIN, J. H.
    1936. IDENTIFICATION, HISTORY, AND DISTRIBUTION OF COMMON SORGHUM
        VARIETIES. U. S. Dept. Agr. Tech. Bul. 506, 102 pp., illus.
(13) WADLEY, F. M.
    1931. ECOLOGY OF TOXOPTERA GRAMINUM, ESPECIALLY AS TO FACTORS
        AFFECTING IMPORTANCE IN THE NORTHERN UNITED STATES.
        Ent. Soc. Amer. Ann. 24: 325–395, illus.
(14) WINTER, J. D.
    1929. A PRELIMINARY ACCOUNT OF THE RASPBERRY APHIDS. Minn. Agr.
        Expt. Sta. Tech. Bul. 61, 30 pp., illus.

EFFECT OF NITROGEN, PHOSPHORUS, AND POTASSIUM ON SUSCEPTIBILITY OF TOMATOES TO ALTERNARIA SOLANI [1]

By H. Rex Thomas

Associate pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture

INTRODUCTION

An important disease of tomatoes (*Lycopersicon esculentum* Mill.) is caused by *Alternaria solani* (Ell. and G. Martin) L. R. Jones and Grout. This disease, which attacks the stem, leaf, and fruit of tomato, is particularly serious on seedlings grown in the Southern States for setting the northern canning acreage. In such districts in the North this fungus often causes a severe leaf blight and frequently an appreciable amount of stem-end infection of the fruit. It has been commonly observed that the disease appears to be worse on plants located in the less fertile parts of a field, on earlier transplanted plants, and on earlier maturing varieties. Moore and Thomas (*3*) [2] found that the infection of tomato seedlings by *A. solani* increases with age. Horsfall and Heuberger (*2*) and Barratt and Richards (*1*) reported that a direct relation exists between yield and defoliation. Thomas (*5*) found that young tomato seedlings grown in the greenhouse in sand with a high nitrogen supply were consistently less susceptible to the collar rot type of stem canker caused by *A. solani* and had smaller lesions than those raised in medium- or low-nitrogen solutions. Horsfall and Heuberger (*2*) found less infection on field-grown tomatoes that received sodium nitrate. They believed that the reduction was due to an overvegetative condition of the plant accompanied by a poor fruit set. Barratt and Richards (*1*) reported that the results of culture tests of various levels of nitrogen in greenhouse sand after artificial inoculation were inconclusive.

The present paper presents the data obtained in 1941 and 1942 from three greenhouse experiments and one field experiment in which the influence of different levels of nitrogen (N), phosphorus (P), and potassium (K) nutrition and their combination on the susceptibility of tomatoes to infection by *Alternaria solani* was studied.

MATERIALS AND METHODS

GREENHOUSE EXPERIMENTS

The variety Indiana Baltimore was used in all the experiments. The greenhouse tests were made at La Fayette, Ind., during fall and spring when the amount of light was sufficient to produce suitable growth.

The plants were grown in quartz sand, 1 part flint grade to 5 parts diamond grade, [3] and were supplied with a complete nutrient solution until they were approximately 8 inches tall. They were then transplanted to fresh sand in 6-inch white glazed pots in the first series and to 2-gallon white glazed crocks in the last two; different nutrient solutions were supplied to the plants. This procedure was followed in order to obtain plants of approximately the same size, but deficient in different amounts of the respective nutrient elements. The plants were transplanted to clean sand and containers before they were given the different treatments, because of the difficulty of removing all of the residues of nutrients applied during the preinoculation period. Four one-plant replicates on each nutrient solution were arranged in randomized blocks on the greenhouse bench.

The sand medium was flushed daily with the solution, the amount being increased with the age of the plant. Distilled water was used in the first series, but tap water was used in the other two. Additional water when needed was supplied during the day. Since the composition of the nutrient solution varied slightly in the different series, the formulas are presented in the respective descriptions. Changes in the composition of the solution were made when necessary to keep the plants at the desired condition of growth and level of nutrition; for example, a sharp reduction in the amount of sunshine could make a relatively low-N plant become either a medium- or a high-N plant and, conversely, a high-N plant might become low in N. Frequent tissue tests were made according to the methods described by Thornton, Conner, and Fraser (7) to determine qualitatively the available nitrogen, phosphorus, and potassium. An effort was made to regulate the low levels of nutrients so that there would be no severely stunted plants. When the plants on the complete preinoculation solution were approximately in the 11- to 12-leaf stage, they were inoculated with a culture of *Alternaria solani* that produced numerous conidia (6). The conidia were washed from 8-day-old petri-dish cultures and suspended in water. A De Vilbiss atomizer operated at about 15 pounds' pressure was used to apply the suspension. After inoculation the plants were kept moist for 48 hours at a temperature of 70° to 75° F. Incubation for longer periods resulted in such severe infection that differences were less apparent. One plant from each nutrient treatment sprayed with water alone served as a check.

When possible the temperature of the greenhouse was kept at approximately 75° F. during the day and at 70° at night. In each experiment there was added to each of the different solutions 2 millimoles of $MgSO_4.7H_2O$ (48.6 p. p. m. of Mg), 5 p. p. m. of Fe, 0.5 p. p. m. of Mn, 0.05 p. p. m. of Cu, 0.05 p. p. m. of Zn, and 0.5 p. p. m. of B. The pH was adjusted to approximately 6 by using HCl or NaOH.

[3] Obtained from Ottawa Silica Co., Ottawa, Ill.

Fruit was allowed to form in the first experiment, but not in the second or third.

Susceptibility of the plants was based on the size of the leaf spots and the number of dead leaves occurring within a certain period after inoculation. The largest diameter was used in estimating the size of a leaf spot. In the first and third experiments the estimated sizes were grouped into four classes: (1) Less than 1 mm.; (2) 1 to 1.9 mm.; (3) 2 to 2.9 mm.; and (4) 3 mm. and larger. In experiment 2 the first three classes were the same, but the fourth represented spots 3 to 3.9 mm. and a fifth those 4 mm. and larger.

Statistical calculations were made according to methods of Snedecor (*4*). Significant differences are indicated by odds of 19:1 (the 5-percent level of *t*). The averages presented in the tables were rounded off from the original data, and the same values will not necessarily be obtained by averaging these rounded-off values.

Experiment 1 was conducted from August 15 to December 17, 1941. Two sets of plants were grown in sand supplied with 12 different nutrient solutions (see table 2) comprising all combinations of 3 levels of N, 2 of P, and 2 of K. One set of 48 plants received only these nutrients throughout the study. A second set of plants was similarly treated until the time of inoculation; then the differential treatments were discontinued and a solution high in N, P, and K was supplied to all plants until the end of the experiment. The first set is described as receiving "continued" treatment and the second as receiving "changed" treatment.

During the preinoculation period the low-N plants received 28.0 p. p. m. of N from $Ca(NO_3)_2.4H_2O$, the medium-N 84.0, and the high-N 252.1. The low-P plants received no P, and the high-P ones received 93.1 p. p. m. from KH_2PO_4 or $Ca(H_2PO_4)_2.H_2O$ depending on whether the chemical was in combination with low or high K. The low-K plants were not supplied with K because of the reserve already present in them, and the high-K plants received 351.9 p. p. m. of K from KCl or KH_2PO_4 depending on the phosphate level of the treatment. Additional $CaCl_2$ was added to maintain the calcium level at 841.5 p. p. m.

When the inoculations were made on November 21, 1941, the medium-N plants were closer in appearance to the low-N plants than to the high-N ones. The low-K plants, although not testing low according to tissue tests, were much smaller than the high-K ones. The high-N, high-P, high-K plants tested low in available phosphate several times during the progress of the experiment, but no deficiency symptoms were observable in the leaves. This indicates that not enough P had been added to supply the plants on this treatment. The susceptibility of these particular plants might have been different if they had been high in P throughout the experiment. Ten days after inoculation the sizes of the leaf spots on all the leaves were determined, and 22 days after inoculation living and dead leaves per plant were counted. The significant difference listed for the changed plants was obtained by using the data for the entire experiment and that for the continued ones by using the continued data only. The latter figure was calculated in order to make possible a more accurate comparison of treatments within the continued part of the experiment.

Experiment 2 was conducted from January 21 to April 29, 1942. Two levels each of N, P, and K in all combinations were supplied to the plants, a total of eight different treatments. The composition of the nutrient solution in parts per million was low-N 84.0 and high-N 280.2 (added as $NaNO_3$); low-K 78.2 and high-K 312.8 (added as KCl); and low-P 7.8 and high-P 31.0 (added as $Ca(H_2PO_4)_2.H_2O$). Calcium chloride was added in a quantity necessary to maintain the calcium level at 400.8 p. p. m. Sodium chloride was added in a quantity necessary to maintain similar osmotic pressures among the treatments and to keep the sodium level at approximately 459.9 p. p. m. During the experiment the intended high-P plants became low in available P, according to plant-tissue tests. Apparently the amount of P added once daily was not sufficient to maintain an excess in the plant tissues during periods of rapid growth. From March 4 the sand cultures were flushed twice daily with their respective solutions. Inoculations were made March 9.

The average size of the leaf spots on the fifth leaf above the cotyledons 10 days after inoculation and the average percentage of leaves dead per plant 28 days after inoculation were determined (see table 3).

TABLE 1.—*Composition of nutrient solutions, experiment 3*

Treatment	Amount of 1-molar solution per liter [1]							
	$NaNO_3$	KNO_3	NaH_2PO_4	KCl	NaCl	$CaCl_2$	$MgSO_4$	$Ca(NO_3)_2$
	Milliliters	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*	*Milliliters*
Low nitrogen:								
Low phosphorus and low potassium	1	1	0	0	3	5	2	0
Low phosphorus and medium potassium	0	2	0	1	3	5	2	0
Low phosphorus and high potassium	0	2	0	7	5	5	2	0
High phosphorus and low potassium	1	1	2	0	1	5	2	0
High phosphorus and medium potassium	0	2	2	1	3	5	2	0
High phosphorus and high potassium	0	2	2	7	3	5	2	0
Medium nitrogen·								
Low phosphorus and low potassium	7	1	0	0	0	5	2	0
Low phosphorus and medium potassium	5	3	0	0	0	5	2	0
Low phosphorus and high potassium	0	8	0	1	5	5	2	0
High phosphorus and low potassium	7	1	2	0	0	5	2	0
High phosphorus and medium potassium	7	1	2	2	0	5	2	0
High phosphorus and high potassium	0	8	2	1	3	5	2	0
High nitrogen:								
Low phosphorus and low potassium	9	1	0	0	0	0	4	11
Low phosphorus and medium potassium	7	3	0	0	0	0	4	11
Low phosphorus and high potassium	9	9	0	0	0	0	4	7
High phosphorus and low potassium	5	1	2	0	0	0	4	13
High phosphorus and medium potassium	9	3	2	0	0	0	4	10
High phosphorus and high potassium	7	9	2	0	0	0	4	8

[1] Micronutrients added and pH adjusted as described in text (p. 290). For the high-N plants in this experiment the amount of $MgSO_4$, however, was doubled.

In experiment 3, August 5 to December 12, 1942, the plants were grown in sand supplied with 18 different nutrient solutions, represent-

ing all combinations of 3 levels of N and K and 2 of P (table 1). On a parts per million basis the N levels were 28.0, 112.1, and 448.2; P 0 and 62.0; and K 39.1, 117.3, and 351.9.

Ten days after inoculation the sizes of the leaf spots on the sixth and ninth leaves above the cotyledons were determined. Thirteen days after inoculation three leaves having spots less than 1 mm. in diameter were tagged on each plant, and 21 days later the average size of these leaf spots was determined. The living and dead leaves per plant were counted 34 days after inoculation (see table 4).

<p style="text-align:center">FIELD EXPERIMENTS</p>

In 1942 a study was made of the influence of different levels and combinations of N, P, and K fertilization of tomato plants on their susceptibility to *Alternaria solani* infection in the field. Part of a field with a Crosby silt loam of low fertility and another part of the same field with a Brookston silty clay loam of high fertility were selected for the test. All combinations of low and high levels of N, P, and K were used—a total of 8 treatments. There were 4 replicates on the Crosby and 4 on the Brookston soil. Each individual plot consisted of 20 plants, 4 plants wide and 5 plants long, set 3½ by 3½ feet. Records were taken on the 6 plants in the center of the plot.

The high levels of the fertilizers applied to the soil were N (120 pounds per acre as ammonium sulfate), P (400 pounds of P_2O_5 per acre as superphosphate), and K (200 pounds of K_2O per acre as muriate of potash). On the Crosby loam the low levels of N and P were 5 percent of the high levels. No N or P was added to the low-N and low-K plots on the Brookston loam. No K was added to the low-K plot on either soil type. The fertilizer was broadcast on top of the soil and plowed under April 23. The plants, Indiana Baltimore variety, were set May 26.

The plants were sprayed with a water suspension of conidia of *Alternaria solani* in mid-July and again in early August. Defoliation records were based upon the percentage of leaves dead on the 3 main stems of each of the 6 center plants in the plot. The average for the 18 stems was used as the percentage for the plot. The first defoliation counts were made August 13. The second counts were made September 2 on the Crosby plot only. At this time the plants on the Brookston plot were very badly defoliated and there were no observable differences among treatments (see table 5). Early yields per acre were determined August 29.

<p style="text-align:center">GREENHOUSE RESULTS</p>

<p style="text-align:center">SIZE OF LEAF SPOTS</p>

The smallest leaf spots in experiment 1 (continued group) were found on the medium-N, high-P, low-K plants (table 2); in experiment 2 on the low-N, low-P, low-K plants (table 3); and in experiment 3 (sixth leaf) on the low-N, high-P, low-K plants (table 4). The largest leaf spots in experiments 1 and 2 occurred on the high-N, low-P, high-K plants and in experiment 3 (sixth leaf) on the high-N, low-P, medium-K plants. Representative leaves of plants grown on the 18 solutions of experiment 3 are shown in figures 1 to 3.

FIGURE 1.—Leaves (sixth above cotyledons) from tomato plants grown at a low-nitrogen level, showing susceptibility to *Alternaria solani*, experiment 3: *A*, Low phosphorus and low potassium; *B*, low phosphorus and medium potassium; *C*, low phosphorus and high potassium; *D*, high phosphorus and low potassium; *E*, high phosphorus and medium potassium; *F*, high phosphorus and high potassium.

FIGURE 2.—Leaves (sixth above cotyledons) from tomato plants grown at a medium-nitrogen level, showing susceptibility to *Alternaria solani*, experiment 3: *A*, Low phosphorus and low potassium; *B*, low phosphorus and medium potassium; *C*, low phosphorus and high potassium; *D*, high phosphorus and low potassium; *E*, high phosphorus and medium potassium; *F*, high phosphorus and high potassium.
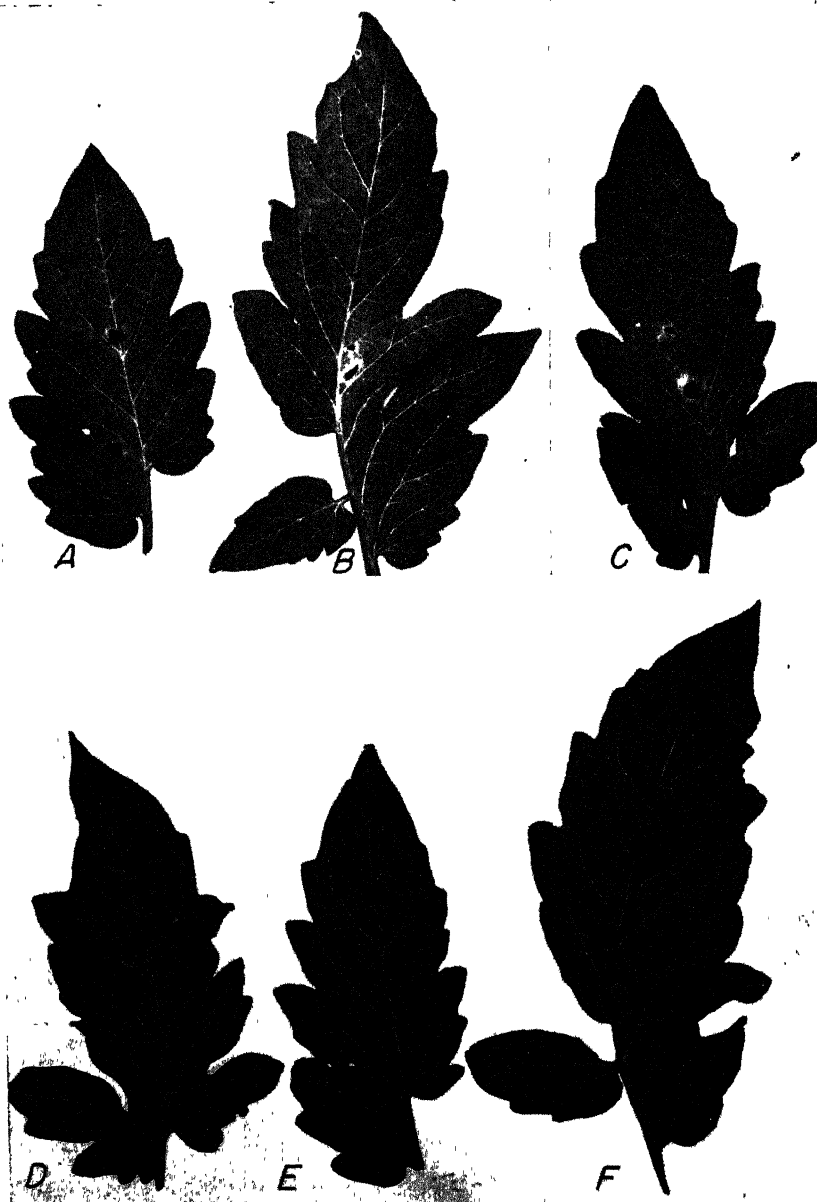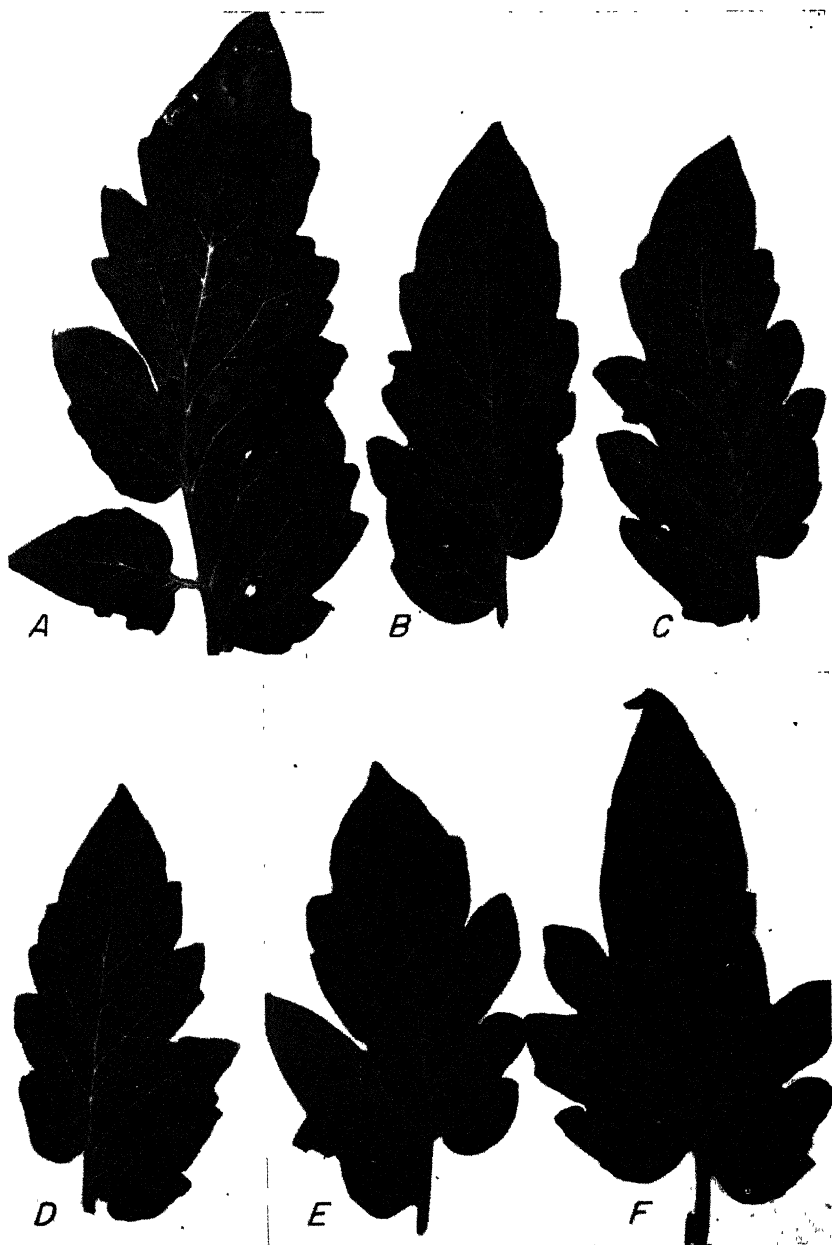
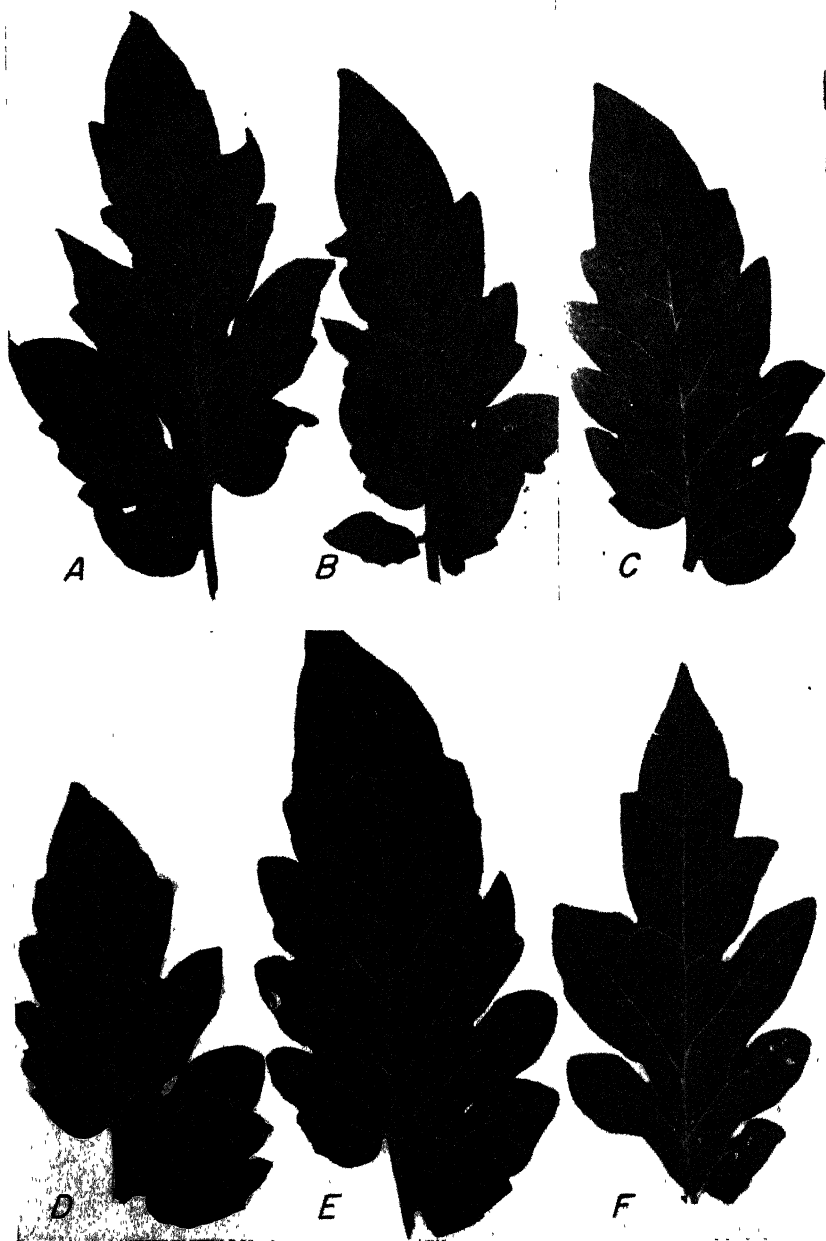FIGURE 3.—Leaves (sixth above cotyledons) from tomato plants grown at a high-nitrogen level, showing susceptibility to *Alternaria solani*, experiment 3: *A*, Low phosphorus and low potassium; *B*, low phosphorus and medium potassium; *C*, low phosphorus and high potassium; *D*, high phosphorus and low potassium; *E*, high phosphorus and medium potassium; *F*, high phosphorus and high potassium.

TABLE 2.—*Susceptibility of tomato leaves to Alternaria solani as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition before and after inoculation, experiment 1*

[Each average based on 4 plants; 10 days after inoculation for size of leaf spots and 22 days after inoculation for dead leaves]

Nutrition treatment before inoculation	Average class [1] for size of leaf spot, with indicated treatment after inoculation		Leaves dead, with indicated treatment after inoculation	
	Continued	Changed [2]	Continued [3]	Changed [2]
			Percent	*Percent*
Low nitrogen:				
Low phosphorus and low potassium	1.1	1.2	14	15
Low phosphorus and high potassium	1.2	1.0	29	34
High phosphorus and low potassium	1 2	1.0	17	10
High phosphorus and high potassium	1.2	1.1	38	27
Medium nitrogen:				
Low phosphorus and low potassium	1.3	1.1	30	11
Low phosphorus and high potassium	1.2	1 1	37	25
High phosphorus and low potassium	1.0	.8	17	9
High phosphorus and high potassium	1 1	1.2	26	19
High nitrogen:				
Low phosphorus and low potassium	1.2	1.2	42	10
Low phosphorus and high potassium	1 6	1.1	53	30
High phosphorus and low potassium	1 1	1.1	13	12
High phosphorus and high potassium	1.3	1.1	10	14
Difference required for significance (5-percent level)	.2	2	16	13
Nitrogen averages:				
Low	1.2	1 1	25	21
Medium	1.1	1.0	28	16
High	1.3	1.1	30	17
Difference required for significance (5-percent level)	.1	.1	8	6
Phosphorus averages:				
Low	1.3	1.1	34	21
High	1 2	1.1	21	15
Difference required for significance (5-percent level)	1	.1	7	4
Potassium averages:				
Low	1.2	1.1	22	11
High	1.3	1.1	32	25
Difference required for significance (5-percent level)	.1	.1	7	4
Treatment after inoculation averages:				
Continued	1.2		27	
Changed	1.1		18	
Difference required for significance (5-percent level)	.1		4	

[1] 1, Less than 1 mm.; 2, 1 to 1.9 mm.; 3, 2 to 2.9 mm.; 4, 3 mm. or more.
[2] Nutrient solution high in nitrogen, phosphorus, and potassium supplied after inoculation of the plants and continued until the end of the experiment.
[3] No leaf spots or dead leaves occurred on uninoculated plants kept throughout the experiment on the same solution (continued treatment).

By averaging all the data for the different N levels it was found that the low- and medium-N plants in the continued group of experiment 1 had significantly smaller spots than high-N plants. In experiment 2 the leaf spots were significantly smaller on the low-N plants. The low-N plants in experiment 3 had significantly smaller leaf spots on the sixth leaf than the medium- and high-N plants. As a group the high-P plants had significantly smaller leaf spots than the low-P in experiments 1 (continued group) and 3. There was no significant difference in size of leaf spots on plants grown at the different P levels in experiment 2. On the average, in experiments 1 and 2 the low-K plants had significantly smaller leaf spots than the high-K ones. There were no significant differences in size of leaf spots on the sixth leaf of plants grown at the three K levels in experiment 3.

On the younger leaves (ninth above the cotyledons, experiment 3) on the differently treated plants there was very little difference in the size of leaf spots.

TABLE 3.—*Susceptibility of tomato leaves to Alternaria solani as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition, experiment 2*

[Each average based on 4 plants; 10 days after inoculation for size of leaf spots and 28 days after inoculation for dead leaves]

Nutrition treatment	Average class [1] for size of leaf spots	Leaves dead [2]
		Percent
Low nitrogen:		
Low phosphorus and low potassium	2 5	30
Low phosphorus and high potassium	3.0	41
High phosphorus and low potassium	2 8	35
High phosphorus and high potassium	3 0	48
High nitrogen.		
Low phosphorus and low potassium	3 8	31
Low phosphorus and high potassium	5.0	54
High phosphorus and low potassium	2.8	14
High phosphorus and high potassium	4 2	35
Difference required for significance (5-percent level)	1.4	13
Nitrogen averages.		
Low	2.8	38
High	3 9	34
Phosphorus averages:		
Low	3 6	39
High	3 2	33
Potassium averages·		
Low	2.9	28
High	3.8	45
Difference required for significance (5-percent level)	.7	7

[1] 1, Less than 1 mm.; 2, 1 to 1.9 mm.; 3, 2 to 2.9 mm.; 4, 3 to 3.9 mm.; 5, 4 mm. or more. All spots on the fifth leaf above the cotyledon were classified.
[2] No leaf spots or dead leaves occurred on uninoculated plants.

In experiment 1 (continued group) there was a significant interaction between N and K. At the low-K level the spots were significantly smaller than at the high-K level on the high-N and low-N plants, but not on the medium-N ones. In experiment 3 a significant interaction between N and P was evident on the sixth leaf above the cotyledons. At the low-P level the leaf spots were larger on the medium- and high-N plants than on the low-N ones, whereas at the high-P level there was little difference in size of leaf spots on the plants at different N levels. A significant interaction existed between N and P and N and K on the ninth leaf above the cotyledons in experiment 3.

Supplying plants previously low in N, P, and K with a high level of them at the time of inoculation retarded the development of leaf spots (table 2).

With one exception the leaf spots on the sixth leaf above the cotyledons were larger than those on the ninth leaf regardless of treatment (table 4). Leaf spots that were less than 1 mm. in diameter 13 days after inoculation increased in 21 days to a range of 1.2 to 2.1 mm., depending upon the nutrient solution supplied the plant. In the 34-day group there were no significant differences among the averages for the N and K levels. The high-P plants had significantly smaller leaf spots than the low-P ones.

TABLE 4.—*Susceptibility of tomato leaves of different ages to Alternaria solani as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition, experiment 3*

[Each average (10 days after inoculation) for columns 2 and 3 based on 4 leaves per plant, for column 4 on 3 leaves per plant (34 days after inoculation), and for column 5 on all leaves (34 days after inoculation)]

Nutrition treatment	Average class [1] for size of leaf spots on indicated leaf			Leaves dead [3]
	Sixth above cotyledon	Ninth above cotyledon	3 per plant [2]	
Low nitrogen:				*Percent*
Low phosphorus and low potassium	1.3	1.1	1.8	19
Low phosphorus and medium potassium	1.2	1.1	1.7	19
Low phosphorus and high potassium	1.2	1.0	1.5	23
High phosphorus and low potassium	1.0	1.0	1.2	12
High phosphorus and medium potassium	1.2	1.0	1.2	11
High phosphorus and high potassium	1.1	1.0	1.4	7
Medium nitrogen:				
Low phosphorus and low potassium	1.4	1.1	1.8	15
Low phosphorus and medium potassium	1.7	1.6	1.7	21
Low phosphorus and high potassium	2.0	1.2	1.8	20
High phosphorus and low potassium	1.6	1.0	1.5	18
High phosphorus and medium potassium	1.1	1.0	1.2	15
High phosphorus and high potassium	1.2	1.0	1.2	11
High nitrogen:				
Low phosphorus and low potassium	1.5	1.2	1.6	16
Low phosphorus and medium potassium	2.3	1.1	2.1	34
Low phosphorus and high potassium	1.8	1.2	2.1	24
High phosphorus and low potassium	1.1	1.0	1.2	4
High phosphorus and medium potassium	1.2	1.1	1.2	2
High phosphorus and high potassium	1.2	1.0	1.2	6
Difference required for significance (5-percent level)	.4	.2	.6	11
Nitrogen averages:				
Low	1.2	1.0	1.5	15
Medium	1.5	1.2	1.5	18
High	1.5	1.1	1.6	14
Difference required for significance (5-percent level)	.2	.1	.3	5
Phosphorus averages:				
Low	1.6	1.2	1.8	22
High	1.2	1.0	1.2	10
Difference required for significance (5-percent level)	.1	.1	.2	4
Potassium averages:				
Low	1.3	1.0	1.5	14
Medium	1.4	1.1	1.5	17
High	1.4	1.1	1.5	17
Difference required for significance (5-percent level)	.2	.1	.3	5

[1] 1, Less than 1 mm.; 2, 1 to 1.9 mm.; 3, 2 to 2.9 mm.; 4, 3 mm. or more.
[2] 13 days after inoculation 3 leaves per plant having spots less than 1 mm in diameter were selected; they were classified for size 21 days later.
[3] No leaf spots or dead leaves occurred on uninoculated plants.

PERCENTAGE OF LEAVES DEAD

The smallest percentage of leaves dead in experiment 1 (continued group) was found on the high-N, high-P, high-K plants (table 2); in experiment 2 on the high-N, high-P, low-K plants (table 3); and in experiment 3 on the high-N, high-P, medium K-plants (table 4). In experiments 1 (continued group) and 2 the largest percentage of leaves dead occurred on the high-N, low-P, high-K plants and in experiment 3 on the high-N, low-P, medium-K plants. Representative plants grown on the 12 solutions of experiment 1 are shown in figures 4 to 6.
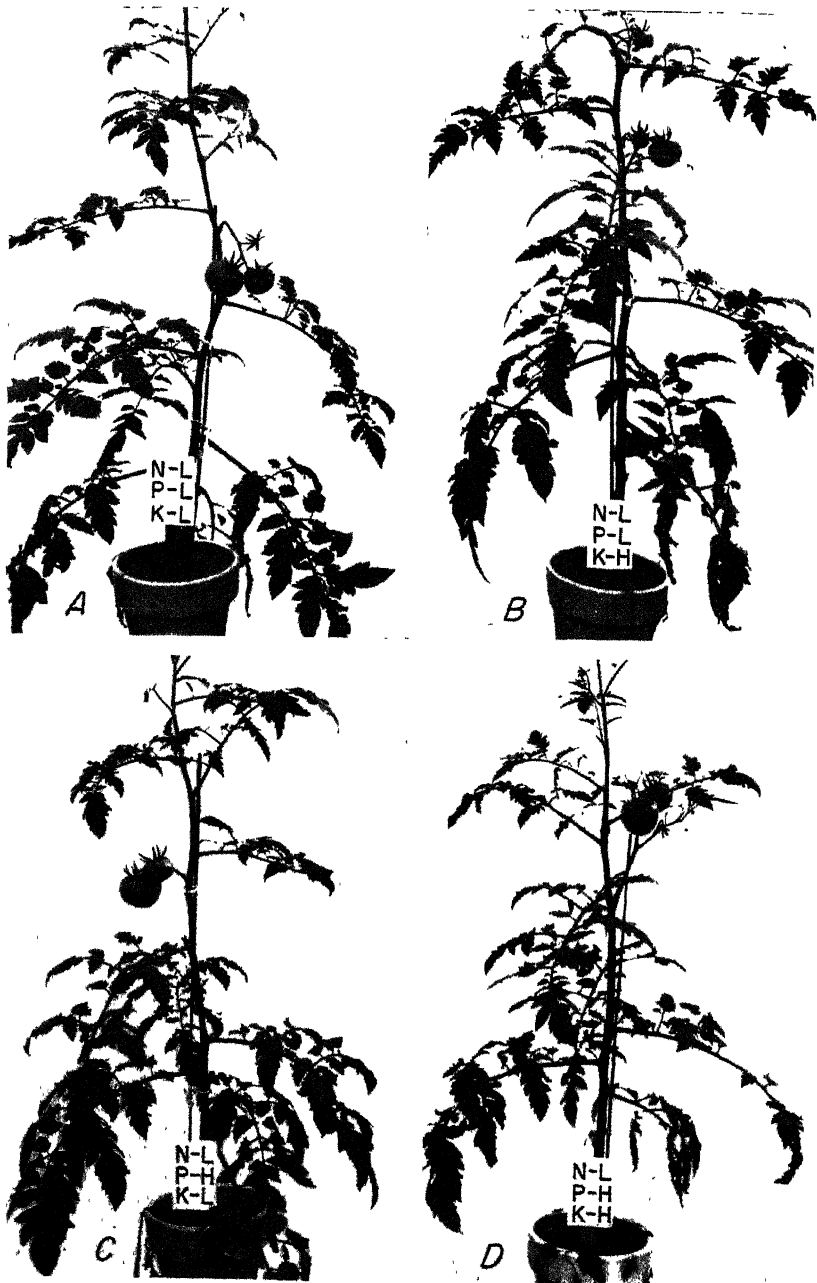
FIGURE 4.—Tomato plants grown at a low-nitrogen level, showing susceptibility of leaves to *Alternaria solani*, experiment 1: *A*, Low phosphorus and low potassium; *B*, low phosphorus and high potassium; *C*, high phosphorus and low potassium; *D*, high phosphorus and high potassium.

FIGURE 5.—Tomato plants grown at a medium-nitrogen level, showing suscepti-
bility of leaves to *Alternaria solani*, experiment 1: *A*, Low phosphorus and low
potassium; *B*, low phosphorus and high potassium; *C*, high phosphorus and
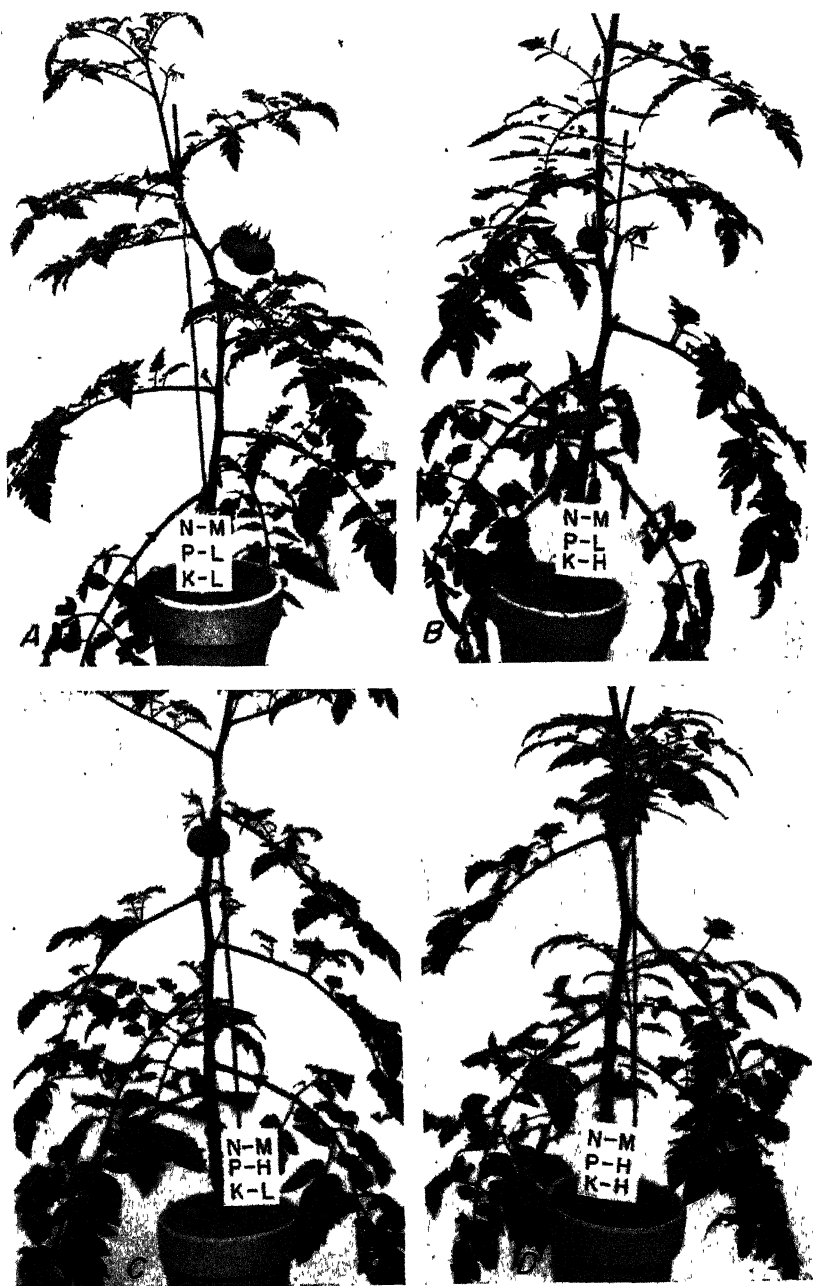low potassium; *D*, high phosphorus and high potassium.

FIGURE 6.—Tomato plants grown at a high-nitrogen level, showing susceptibility of leaves to *Alternaria solani*, experiment 1: *A*, Low phosphorus and low potassium; *B*, low phosphorus and high potassium; *C*, high phosphorus and low potassium; *D*, high phosphorus and high potassium.

By averaging the data for the different N levels it was found in all three experiments that there was no significant difference in the percentage of leaves dead. In experiment 1 (continued group) and experiment 3 the high-P plants had a significantly smaller percentage of leaves dead than the low-P ones. There were no significant differences in percentage of leaves dead at the different P levels in experiment 2. The low-K plants had a significantly smaller percentage of leaves dead than the high-K ones in experiments 1 (continued group) and 2. In experiment 3 there were no significant differences in percentage of leaves dead at the different K levels.

In the continued group of experiment 1 and in the other experiments there was a significant interaction between N and P. In experiments 1 and 2 on the low-N plants there was more defoliation at the high-P level than at the low-P, whereas the reverse occurred at the other N levels. In experiment 3 the percentage of leaves dead, although less on the high-P than on the low-P plants at all N levels, was much smaller at the high-N level than at either the medium- or the low-N level. In experiment 1 there was a significant interaction between N and K. At the high-K level there was a proportionately greater percentage of leaves dead on the low-N plants than on the medium- and high-N ones.

Supplying plants previously low in N, P, and K with them at the time of inoculation reduced the percentage of leaves dead (table 2).

FIELD RESULTS

On August 13 there were no significant differences in percentage of leaves dead on plants grown on the different soil types, so that averages for the two soils can be compared in studying results for that date. The most defoliation occurred on the low-N, low-P, high-K plots and the least on the high-N, high-P, low-K ones (table 5).

On the average, significantly less defoliation occurred on the high-N plants than on the low-N ones. It is recognized that part of the defoliation on the low-N plants may have been due to nutritional effects aside from those involving attacks by *Alternaria*. There were no significant differences between P or K levels.

Records taken September 2 show that the amount of defoliation, 72 to 90 percent, is so great that any slight differences that existed are of no practical significance.

In the field no practical differences in the percentage of leaves dead existed between the treatments late in the season. This was particularly true on the Brookston silty clay loam of high fertility, where the plants were large and conditions were favorable for the increase of the fungus. The greater defoliation on the low-N plants, particularly noticeable early in the season, may have been due more to the early development of a relatively heavy crop of fruit than to the direct effect of nitrogen on disease development. This might also be the explanation for the greater defoliation of the high-P than of the low-P plants late in the season.

TABLE 5.—*Susceptibility of field-grown tomato plants to infection by Alternaria solani on Crosby and Brookston soil series as influenced by different levels and combinations of nitrogen, phosphorus, and potassium nutrition*

[Each soil-type average based on 4 replicates of 6 plants each]

Nutrition treatment	Leaves dead (Aug. 13) when grown on indicated soil type			Leaves dead when grown on Crosby loam (Sept. 2)	Early yield per acre (Aug. 29) [1] when grown on indicated soil type		
	Crosby	Brooks-ton	Both		Crosby	Brooks-ton	Both
	Percent	*Percent*	*Percent*	*Percent*	*Tons*	*Tons*	*Tons*
Low nitrogen:							
Low phosphorus and low potassium	32	32	32	84	8.0	7.6	7 8
Low phosphorus and high potassium	34	33	33	72	8.4	8 8	8 6
High phosphorus and low potassium	26	29	28	85	9.6	9.4	9 5
High phosphorus and high potassium	24	30	27	90	9.0	8.2	8.6
High nitrogen·							
Low phosphorus and low potassium	18	17	17	76	6.3	6.8	6.6
Low phosphorus and high potassium	22	22	22	78	7.2	7.0	7 1
High phosphorus and low potassium	12	18	15	85	6.2	7.9	7.0
High phosphorus and high potassium	18	28	23	74	6 3	7.4	6.8
Difference required for significance (5-percent level)	13	13	9	8	2.3	2.3	1 6
Nitrogen averages:							
Low	--------	--------	30	83	--------	--------	8.6
High	--------	--------	19	78	--------	--------	6.9
Phosphorus averages·							
Low	--------	--------	26	78	--------	--------	7.5
High	--------	--------	23	84	--------	--------	8.0
Potassium averages.							
Low	--------	--------	23	82	--------	--------	7.7
High	--------	--------	26	78	--------	--------	7.8
Difference required for significance (5-percent level)	--------	--------	5	4	--------	--------	1.1

[1] No additional yields were obtained.

DISCUSSION

The data presented show that the degree of injury caused by *Alternaria solani* infection of tomato foliage was influenced by the supply of N, P, and K. The reactions to the various levels and combinations of these elements differed somewhat in each of the three greenhouse experiments. The differences may have been due to variations in the composition of the plant caused by fluctuations in the amount of sunlight during the growing period, in the environment during the period of disease development, or in the composition of the nutrient solutions. In experiments 1 and 2 the supply of P was not adequate at times to maintain as high a level as was desired in the high-N, high-P, high-K plants.

Plants receiving certain treatments reacted to infection similarly in all three series as well as in several experiments not described herein. The plants grown on solutions medium to high in N, high in P, and low to high in K were injured least by *Alternaria solani*. In several instances the low-N, low-P, low-K plants were also damaged little. Plants grown on solutions of medium- to high-N, low-P, medium- to high-K level were usually the most severely injured.

There probably would have been fewer dead leaves on the high-N, high-P plants in experiments 1 and 2 if a higher level of P had been maintained throughout the experiment.

In general the susceptibility of the plants as indicated by the size of the leaf spots agreed with that indicated by the percentage of leaves dead. The susceptibility of the plants as measured by the number and size of stem cankers (data not presented) did not correlate so closely.

In all three series the percentages of leaves dead indicated that there was a significant interaction between N and P. The expression of the interaction, however, was not always consistent. In experiments 1 (continued group) and 2 at the low-N level the high-P plants had more defoliation than the low-P plants. In experiment 3 the reverse occurred. In every case the high-N, low-P plants had more defoliation than those grown with other N and P combinations and the high-N, high-P plants always had the least. Observing the over-all effects of N, P, and K, respectively, obscures the effects of interaction between these elements.

The mechanism responsible for the differences in reaction to disease development is unknown. The rapid collapse of leaf tissue about the point of infection on the susceptible plants suggests that a toxin may be formed in greater amounts in leaf tissue of the type developing on plants of a particular nutrient condition or may diffuse more rapidly through it. Whipple (8) reported that a toxin is produced by *Alternaria solani* and diffuses into the plant tissue for 12 cm. or more in advance of the mycelium. The apparent effect of toxin ahead of the mycelium has also been noted by Thomas.[4]

The degree of resistance exhibited under the controlled conditions in the greenhouse would probably be of limited value in the field. Infection of new growth in the field occurs at such a rapid rate under suitable weather conditions that any differences that might exist in the sizes of leaf spots would be concealed by the abundance of infection. However, plants with adequate nutritional supply are able to continue growing and develop new shoots in the center throughout the season and thus protect the fruit from sun injury. This type of protection is satisfactory until a period of weather ideal for heavy infection again occurs.

SUMMARY

Varying the amounts and combinations of N, P, and K in sand-culture greenhouse tests affected the response of tomato plants to infection by *Alternaria solani*.

By averaging all the data for the different P levels it was found that in two of the three experiments the plants grown at the high level of P, irrespective of N and K levels, had smaller leaf spots and a significantly smaller percentage of leaves dead than the plants grown at the low-P level.

By averaging all the data for the different N levels it was found that in each of the three experiments plants grown at the low level of N, irrespective of P and K levels, had significantly smaller leaf spots than those at the high-N level. There was no significant difference in the percentage of leaves dead on the plants on the different N levels.

On the average, plants grown at the low K level, irrespective of N and P levels, had a significantly smaller percentage of leaves dead and smaller leaf spots than those grown at the high level in two of the three series.

---

[4] THOMAS, H. R. COLLAR-ROT INFECTION ON DIRECT-SEEDED TOMATOES. U. S. Bur. Plant. Indus., Plant Dis. Rptr. 24 (1): 8–10. 1940. Processed.]

A significant interaction existed between N and P in the percentage of leaves dead in all three greenhouse experiments. In every case the high-N, low-P plants had the largest percentage of leaves dead and the high-N, high-P plants the smallest.

Plants grown on solutions medium to high in N, low in P; and medium to high in K were usually the most susceptible to infection as expressed by the development of leaf spots and percentage of leaves dead. The most resistant plants were grown on solutions medium to high in N, high in P, and low to high in K.

The group of plants that received a nutrient supply high in N, P, and K at the time of inoculation had significantly smaller leaf spots and percentage of leaves dead than the group which continued to receive solutions deficient in N, P, and K after inoculation.

The leaf spots were larger on the older leaves than on the younger ones, regardless of the apparent nutritional condition of the plants.

A limited field fertilizer test in Indiana indicated little if any difference in the disease resistance of plants grown at different levels of N, P, and K and with combinations of them.

LITERATURE CITED

(1) BARRATT, R. W., and RICHARDS, M. C.
     1944. ALTERNARIA BLIGHT VERSUS THE GENUS LYCOPERSICON. N. H. Agr. Expt. Sta. Tech. Bul. 82, 24 pp., illus.

(2) HORSFALL, J. G., and HEUBERGER, J. W.
     1942. CAUSES, EFFECTS AND CONTROL OF DEFOLIATION ON TOMATOES Conn. (State) Agr. Expt. Sta. Bul. 456, pp. [181]–223, illus.

(3) MOORE, W. D., and THOMAS, H. R.
     1943. SOME CULTURAL PRACTICES THAT INFLUENCE THE DEVELOPMENT OF ALTERNARIA SOLANI ON TOMATO SEEDLINGS. Phytopathology 33: 1176–1184, illus.

(4) SNEDECOR, G. W.
     1946. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 4, 485 pp., illus. Ames, Iowa.

(5) THOMAS, H. R.
     [1940.] NITROGEN NUTRITION OF TOMATO SEEDLING AFFECTS SUSCEPTIBILITY TO COLLAR ROT. Ind. Agr. Expt. Sta. Ann. Rpt. (1938–39) 52: 56.

(6) ———
     1943. A NONCHROMOGENIC SPORULATING VARIANT OF ALTERNARIA SOLANI. Phytopathology 33: 729–731, illus.

(7) THORNTON, S. F., CONNER, S. D., and FRASER, R. R.
     1936. THE USE OF RAPID CHEMICAL TESTS ON SOILS AND PLANTS AS AIDS IN DETERMINING FERTILIZER NEEDS. Ind. Agr. Expt. Sta. Cir. 204, 16 pp., illus. (Revised.)

(8) WHIPPLE, O. C.
     1938. MACROSPORIUM SEEDLING BLIGHT OF TOMATOES. Wis. Univ. Sums. Doctoral Diss. 3: 65–66.

REACTION OF ALFALFA VARIETIES, SELECTIONS, AND HYBRIDS TO ASCOCHYTA IMPERFECTA [1]

By L. P. Reitz, *agronomist, Kansas Agricultural Experiment Station;* C. O. Grandfield, *associate agronomist, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture;* M. L. Peterson, G. V. Goodding, M. A. Arneson, *formerly graduate research assistants;* and E. D. Hansing, *associate plant pathologist, Kansas Agricultural Experiment Station*

INTRODUCTION

Black stem caused by *Ascochyta imperfecta* Peck is an important disease of the leaves and stems of alfalfa (*Medicago sativa L.*). It is especially destructive in cool, wet seasons and in humid regions. In Kansas it causes greatest damage to the spring growth. In years of heavy infection, the yield may be reduced and the feeding quality lowered, especially as defoliation progresses. In severe cases the stands of alfalfa are made thin or killed entirely, but this is not the usual result from infection at this station. The disease has been reported in many of the alfalfa-growing States of the United States and in some other countries.

Peterson and Melchers [2] reviewed the literature and reported research on the characteristics of the disease, identification of the causal organism, and methods used in artificial inoculation.

Early symptoms of black stem are small, dark-brown or black spots on the leaves and stems. As the lesions enlarge and coalesce the leaves become chlorotic and drop from the plant. Stem lesions turn black and coalesce, causing the stem surface of infected areas to blacken and sometimes to die. The portions nearest the ground usually are most severely infected. Greenhouse inoculations with spore suspensions of the fungus produced typical lesions on the leaves and stems, but the most uniform infection was obtained on the leaves.

It is the purpose of this paper to present the data on genetic differences in susceptibility to the fungus observed in experiments conducted at Manhattan, Kans., during the years 1938 to 1943, inclusive.

MATERIAL AND METHODS

Observations on susceptibility of varieties and strains of alfalfa to black stem were made in the field and greenhouse. In the field, plots sown for yield determinations or demonstrations were used. Green-

[2] Peterson, M. L., and Melchers, L. E. studies on black stem of alfalfa caused by ascochyta imperfecta. Phytopathology 32:590–597, illus. 1942.

Journal of Agricultural Research,
Washington, D. C.

Vol. 76, No. 12
June 15, 1948
Key No. Kans.–103

(307)

house plantings were made in unglazed clay pots. Usually one plant was grown in each pot. The plants were inoculated by spraying them with a suspension of spores, after which they were placed in a canvas-covered moist chamber and kept there for 72 hours.

Considerable time was spent in finding a suitable method of scoring the plants for reaction to black stem. Classes of resistance formed an intergrading series and immunity from disease was not observed in any variety or strain. Several somewhat different methods were used, but high correlations in the results of the different methods were noted. In most of the greenhouse tests disease scores for individual plants were determined by ratings on the 0 to 5 or 0 to 10 basis. Plants were scored for (1) number of leaf lesions, (2) size of leaf lesions, (3) defoliation, (4) petiole lesions, and (5) stem lesions. These can be transferred to a basis of 100 by multiplying each score by 10 or 20 depending upon the basis of scoring and the numbers averaged for a plant score. Typical leaves showing degrees of infection are shown in figure 1.
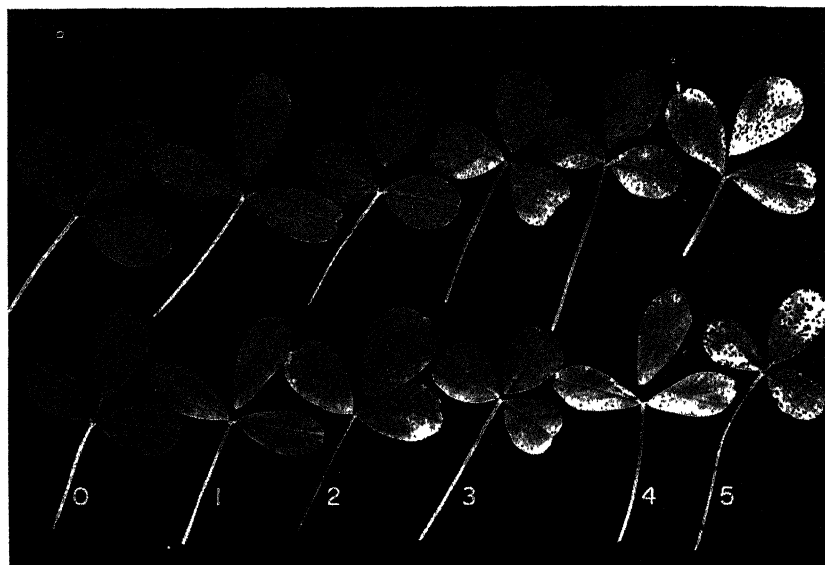

FIGURE 1.—Typical leaves of alfalfa showing grades of black stem infection on a scale of 0 to 5. Leaves in upper row show increasing numbers of lesions; those in lower row show different sizes of lesions.

By artificial inoculation and with a moist chamber, many plants grown in the greenhouse could be tested. When this was done, each group of plants was accompanied by four check plants from a clonally propagated plant of Ladak alfalfa. By scoring the check plants an index of infection could be calculated and different groups compared. This was the most satisfactory means of comparing lots inoculated at different times. A sample calculation follows:

(1) Number of leaf lesions_____ 6×10=60
(2) Size of leaf lesions_____ 4×20=80
(3) Defoliation_____ 7×10=70
(4) Petiole lesions_____ 3×20=60
(5) Stem lesions_____ 1×20=20

Total_____ __ _____ 290

290÷5=58 the average plant score.

If the score of check plants was 48, the index number of infection of this plant would be 58÷48×100 or 121.

Richards[3] devised a system of scoring plants in the field. A sample consisted of all the stems which were within a ring cast into a plot. Enclosed stems were separated into six classes depending upon the degree of severity of the disease, with 1 as the class of least severity. The number of stems was multiplied by the class number and the sum total thus derived was divided by the total number of stems collected. This method was satisfactory under field conditions when infection on the stems was heavy, but was not so good for mild infection or during early stages of development. A modified system was devised in the present study which gave weight to leaves and stems separately. Such values could be combined for a plant score, and scores could be changed to percentages as desired.

Isolations of the fungus were made from several collections to study cultural characteristics and differences in pathogenicity.

The analysis of variance, in conjunction with associated tests of significance, afforded the basis for most of the statements contained in this paper on statistical differences between units or treatments. Several other statistical methods also were used to reduce the large masses of data recorded. In general, these are mentioned when different experiments are discussed.

EXPERIMENTAL RESULTS

VARIETAL REACTION UNDER FIELD CONDITIONS

Johnson and Valleau[4] indicated that resistance to black stem was inherent in some varieties of alfalfa. Toovey, Waterston, and Brooks[5] gave the results of black stem readings on alfalfa strains in which "the strains were placed in the following order of decreasing susceptibility in 1935: (1) Medanos, (2) English grown and Grimm, (3) Provence, Marlborough and Hungarian." Richards[3] made detailed readings on 44 varieties during a severe epidemic in 1933, and found the disease to be least severe in Ladak and most severe in a French introduction. Introductions from Russia and Turkistan were severely infected; Grimm and Hardigan were less damaged. Peterson and Melchers[6] reported varietal and species differences.

[3] RICHARDS, B. L. REACTION OF ALFALFA VARIETIES TO STEM BLIGHT. Phytopathology 24: 824–827, illus. 1934.
[4] JOHNSON, E. M., and VALLEAU, W. D. BLACK-STEM OF ALFALFA, RED CLOVER AND SWEET CLOVER. Ky. Agr. Expt. Sta. Bul. 339: 55–82, illus. 1933.
[5] TOOVEY, F. W., WATERSTON, J. M., and BROOKS, F. T. OBSERVATIONS ON THE BLACK-STEM DISEASE OF LUCERNE IN BRITAIN. Ann. Appl. Biol. 23: 705–717, illus. 1936. (See p. 707.)
[6] See footnote 2, p. 307.

Varietal differences in susceptibility were found in preliminary trials at Manhattan in 1939 from four sets of data involving three methods of making disease readings. Differences between varieties in each experiment were statistically significant, indicating the possibility of breeding for resistance. Turkistan varieties were more susceptible than Ladak or Kansas Common in these trials.

The spring of 1942 was exceptionally cool and wet; thus climatic conditions were favorable both for plant growth and for infection with black stem in the field. Readings were made on 66 strains of alfalfa planted in 20-foot rows with two replications in the uniform nursery on the agronomy farm at Manhattan. The nursery contained improved strains from Rhode Island, New Jersey, New York, Michigan, Wisconsin, Nebraska, Kansas, and Colorado as well as some of the standard varieties, such as Ladak, Kansas Common, Grimm, Meeker Baltic, Orestan, Hardistan, Dakota Common, and Arizona Chilean. In addition, a few foreign introductions were grown. Notes on black stem were taken on April 30, or about a month before the crop was cut for hay. At that time black stem was the predominant disease, but 4 weeks later yellow leaf blotch caused by *Pseudopeziza jonesii* Nannf. was more abundant than black stem. Severe defoliation had occurred from the time of the first reading and a large share of this was attributed to black stem. Analysis of variance of the black stem readings showed that the variation due to strains was highly significant, far exceeding the 1-percent level, thus indicating a real difference among the strains. A–169 from Nebraska, A–155 from Rhode Island, and 128 and A–131 from Wisconsin were the most resistant strains according to these readings. All of the foreign introductions and Arizona Chilean were very susceptible. Of the standard varieties, Grimm and Dakota Common ranked highest in resistance.

OBSERVATIONS IN GREENHOUSE TESTS

A comprehensive test of 45 artificially inoculated plants of each of 10 varieties was made in the greenhouse. The purpose of this experiment was (1) to test the reliability of readings on artificially inoculated plants, and (2) to determine whether improvements could be made by selecting plants in this way. The 10 varieties were Turkistan 86696, Turkistan 19304, Ladak, Kansas Common, Kansas Common Selection, Grimm, Hairy Peruvian 22486, *Medicago falcata* F. C. 30114, *Medicago ruthenica* F. P. I. 190365, and Semipalatinsk F. C. 22613. Seed was planted in flats in the greenhouse on May 17, and on June 22 the seedlings were transplanted to rows in the irrigated nursery. On October 2, 5 plants from each variety were dug, transplanted into 7-inch pots, and brought into the greenhouse. Five days later a second group of 50 plants was brought into the greenhouse. This procedure was repeated every 5 days until 450 plants had been potted.

By November 28 the first group of plants was of sufficient size to be inoculated. Inoculations were made at regular intervals so that the foliage on all groups of plants was approximately the same age when the plants were inoculated. Two readings were made on each plant. The first was made 8 to 10 days after inoculation and the second 5 days later. A summary of the readings is given in table 1. The disease score for the whole plant is the sum of the scores for

stems and leaves estimated on a 0 to 10 basis.   Clonally propagated check plants were not used in this test.

TABLE 1.—*Average disease readings and rank of 45 plants from each of 10 alfalfa varieties, showing coefficients of variation for size and number of lesions*

Variety	Whole plant		Score of—		Size of lesions		Number of lesions	
	Score	Rank	Stems	Leaves	Score	Coefficient of variation	Score	Coefficient of variation
Medicago ruthenica	2.38	1	0 42	1 96	2.11	49.7	1.80	57.5
Semipalatinsk	3 39	2	.58	2.81	3.18	36.9	2.44	45 8
Ladak	3.47	3	.53	2.94	3 33	27.1	2.56	54.4
Grimm	3.93	4	.78	3.15	3.44	24.4	2.87	47 4
Medicago falcata	4 08	5	.91	3.17	3.44	34.2	2.89	49 1
Turkistan 19304	4 36	6	.80	3.56	3.58	28.3	3 53	36 5
Turkistan 86696	4 37	7	.87	3.50	3.71	26.1	3.29	41.8
Kansas Common	4.71	8	1 15	3 56	3.80	26.7	3.31	42.1
Kansas Common Selection	4.93	9	1.35	3.58	3 93	23.6	3.22	45 9
Hairy Peruvian	6.19	10	2.02	4.17	4 44	17.0	3.89	26.9
Average	4 08		.94	3.24	3. 50	29.4	2.98	44.7

In this experiment, the disease scores ranged from 2.38 for *Medicago ruthenica*, the most resistant variety, to 6.19 for Hairy Peruvian, which was extremely susceptible.   The Turkistans and Kansas Commons ranked higher than average in susceptibility.   The fact that Kansas Common scored higher in susceptibility than Turkistan is important, since disease readings in the field under natural conditions for infection have consistently shown Turkistan types to be the more heavily diseased.   These results suggest that the two varieties may react differently under conditions of natural and artificial infection. Differences in disease readings for varieties were highly significant.

Stems showed rather poor infection from artificial inoculation; however, differences between variety means were significant beyond the 1-percent point.   Leaf readings showed heavier infections than those for stems with a wider range in scores.   The rank of the different varieties for stem and leaf scores corresponded very closely.   Thus it appeared that factors conditioning stem infection were the same as those conditioning leaf infection.

The leaf score was obtained by giving equal but separate consideration to size of lesions and number of lesions.   There were two reasons for considering these two factors independently: (1) A plant with few but very large lesions would be damaged as severely as a plant with more numerous but small lesions and (2) preliminary inoculations had shown that plants varied considerably in respect to size and number of lesions.   The score for size of lesions ranged from 2.11 on *Medicago ruthenica* to 4.44 on Hairy Peruvian.   The differences were highly significant.

The score for number of lesions on the leaves ranged from 1.80 on *Medicago ruthenica* to 3.89 on Hairy Peruvian and the differences between variety means were highly significant.

Comparison of the ranking of the various varieties for size and number of lesions indicated a close relationship in general, although

Turkistan 19304 ranked sixth in size of lesions and ninth in number of lesions while Kansas Common Selection ranked ninth in size of lesions and sixth in number of lesions. These results indicate that there may be two plant characteristics for resistance of leaves to black stem disease, one restricting the entrance of the organism, the other inhibiting its growth once it is inside.

PLANT VARIATION WITHIN VARIETIES

There were wide variations in disease readings within a variety. For example, readings for Kansas Common ranged from 1.5 to 9.0, Turkistan 86696 from 2.0 to 8.0, and Ladak from 1.5 to 8.0. This range included several sources of variation, a part of which was considered heritable. Coefficients of variability were calculated to indicate variation within varieties with respect to size and number of lesions (table 1).

Hairy Peruvian showed the least variability for size of lesions, i. e., it approached complete susceptibility. Kansas Common Selection, Grimm, Turkistan 86696, Kansas Common, Ladak, and Turkistan 19304 followed in increasing order of variability but with only a small range from low to high. *Medicago falcata*, Semipalatinsk, and *M. ruthenica* were considerably more variable. The coefficients for number of lesions also ranked Hairy Peruvian as least variable and *M. ruthenica* as most variable.

REACTION OF 30 PEDIGREE LINES FROM KANSAS COMMON

Ten plants from each of 30 bacterial wilt-resistant selections from Kansas Common were potted in the field and brought into the greenhouse in November 1942. These were young plants from seed planted on August 30. When the plants became 8 to 10 inches high they were selected at random on the basis of height only and inoculated with the fungus. Readings were made in the usual manner, and the scores and indices were calculated. In this case a Turkistan clone was used as the standard check. Considerable variability within lines was found. Since these lines were from open-pollinated plants, much variability was expected because of the well-known heterozygosity in alfalfa. The plants were cut back after the readings had been made on the first inoculation. Many of the plants were blossoming at that time. Four weeks later these plants were inoculated again and readings were made. A highly significant difference existed between inoculations, and the lines were significantly different. Kansas Common line 1–102–5 had the lowest average score of 82 for 7 plants.

Readings from the second inoculation exceeded those from the first in 23 of the 30 lines tested. In the other 7 lines the reverse was true. It is difficult to explain the significant variation between inoculations, as all plants were of the same age and received the same treatment. Some of the variation may have been due to variation in readings and some to the difference in recovery of the plants after cutting, as some plants recovered faster than others. The root reserves may have been higher in the plants when inoculated the second time than the first, as the majority of the plants were blooming or about to bloom

when cut back after the first inoculation. Therefore, differences in root reserves would be expected. Temperature likewise may have been a contributing factor since greenhouse temperatures were higher during the early part of the season than later. Other factors not known at present may also have contributed to this difference between inoculations.

Analysis of variance showed that significant differences were present between lines, but there was so much variation within lines that the 22 highest ranking ones did not differ significantly.

REACTION OF PLANT SELECTIONS AND INBRED LINES

Parental stocks for inbreeding were selected from plants used in the variety tests reported above with the exception of the 30 bacterial wilt-resistant selections. At least 1 resistant and 1 susceptible plant were chosen from each variety. The terms "resistant" and "susceptible" as used here, refer to the relative resistance of plants within each variety. Thus, a resistant plant from a relatively susceptible variety might have been more susceptible to the disease than a susceptible plant from a more resistant variety.

The selected plants were self-pollinated in the greenhouse in an insect-free room. Enough seed was gathered from each plant to insure a progeny class of at least 27 plants. The seeds were scarified and planted in flats. When seedlings had attained about 4 inches of growth, they were transferred to 6-inch pots, where they were left throughout the tests. Clonally propagated check plants were used as standards for comparison in all inoculation tests.

Inbred plants were allowed to develop a vigorous growth and were selected for inoculation when they were 10 to 12 inches in height. Plants were chosen at random throughout all of the varieties used, with the size of the plant as the only characteristic considered in making selections for inoculation.

A detailed description of the results obtained in testing the inbred plants from the selected parents follows:

Kansas Common variety.—Two plants were used as parents from which inbred lines were established in the variety Kansas Common. Kansas Common No. 6 was a resistant plant and Kansas Common No. 18 was susceptible. Twenty-seven inbred plants from each of these parents were tested. Their plant scores were derived and each plant was given an index of infection in terms of the check plants' score. The two progenies were compared by the use of analysis of variance; the results are presented in table 2.

The means of each group clearly indicate that the progeny of No. 6 was significantly more resistant than the progeny of No. 18. The analysis of variance substantiated this assumption, for it showed that highly significant differences existed between the two inbred lines. The standard deviations of the two groups showed that considerable variation occurred in each population.

Ladak variety.—Four inbred lines were developed in the variety Ladak. Of the four parent plants, No. 11 was resistant and the other three plants, Nos. 20, 5, and 15, were only moderately resistant. On the basis of inheritance, the inbred lines on the average were expected to resemble their parents in resistance. Table 2 shows the differences

that occurred between the progeny of the resistant plant and the progenies of each of the susceptible plants. These data demonstrate the resistance of selection 11 and the similarity of the three progenies from the moderately resistant plants.

TABLE 2.—*A summary of the data on resistance to black stem in the inbred progenies of resistant and susceptible plant selections from 5 varieties and strains of alfalfa*

Variety and selection number	Inbred progenies		Standard deviation
	Mean	Difference	
Kansas Common:			
No. 6	101 3		37.9
No. 18	147.3	46.0**	29.0
Ladak:			
No. 11	89.9		20.4
No. 20	111 6	21.7**	24.9
No. 11	89.9		20.4
No. 5	118.3	28.4**	32.0
No. 11	89.9		20 4
No. 15	105.8	15.9**	22.7
No. 20	111.6		24.9
No. 5	118.3	6.7	32.0
No. 20	111.6		24.9
No. 15	105.8	5.8	22.7
No. 5	118.3		32.0
No. 15	105.8	12.5	22.7
Turkistan:			
No. 36	128.1		10.6
No. 16	113.3	14.8	32.9
No. 36	128.1		10.6
No. 40	144.0	15.9**	18.6
No. 16	113.3		32.9
No. 40	144.0	30.7**	18.6
Hairy Peruvian:			
No. 33	148.8		20.1
No. 5	142.2	6.6	32.6
No. 33	148.8		20.1
No. 13	163.4	14.6*	20.7
No. 5	142.2		32.6
No. 13	163.4	21.2**	20.7
Kansas Common Selection:			
No. 1-9	113.0		20.1
No. 1-6	131.7	18.7*	30.4
No. 1-9	113.0		20.1
No. 1-13	96.8	16.2**	18.8
No. 1-6	131.7		30.4
No. 1-13	96.8	34.9**	18.8

*Significant at 5-percent level.
**Significant at 1-percent level.

Turkistan variety.—Three Turkistan plants were selected for inbreeding. Two of them, Nos. 36 and 16, were moderately resistant, whereas the third one, No. 40, was very susceptible. The progenies of the 2 resistant plants maintained that resistance and showed no significant difference between groups. The progenies from the resistant parents were both significantly more resistant than the

inbreds developed from the susceptible plant No. 40. From Turkistan No. 36 there were only 21 inbred plants, 33 from No. 16, and the usual 27 from No. 40. The data summarized in table 2 indicate that inbreeding in the Turkistan variety results in the production of lines that react, as a group, much the same as their parents

Hairy Peruvian variety.—Three plants were selected for inbreeding from the very susceptible variety, Hairy Peruvian. Two of these plants, Nos. 33 and 5, were slightly resistant, and the third, No. 13, was highly susceptible. No significant difference was observed between the progenies of the two slightly resistant parents, as is shown in table 2, but they were significantly more resistant than the progeny of the susceptible parent, No. 13.

Kansas Common Selection.—Three plants of Kansas Common Selection were utilized for inbreeding. These three plants were classed as resistant, moderately resistant, and susceptible. The plant listed as susceptible produced inbred progeny with a higher level of resistance than either of the progenies of the supposedly resistant parents. Apparently the original classification of this plant was in error. The progeny of the plant listed as resistant was significantly more resistant than the progeny of the moderately resistant plant, demonstrating that in one instance, at least, this variety behaved as the other varieties did. On the other hand, some discrepancies were foreseen from the outset, since in early selection work no method was employed to correct for variations that arose between different dates of inoculation. Table 2 shows a summary of the data and comparisons of the differences that existed between the progenies of the plant selections.

Medicago falcata.—Two strains of the species *Medicago falcata* were utilized in the inbreeding studies. Both strains had probably been intercrossed to some extent with common alfalfas, but *M. falcata* features were evident in the parental selections, and a high degree of disease resistance, characteristic of the species, prevailed.

Medicago falcata No. 39.—A very resistant *M. falcata* plant was successfully inbred and its progeny proved to carry the same high level of resistance. The mean of the group was 81.61, the highest average resistance of any inbred group tested. The variation within this group was also low, as indicated by a standard deviation of ±20.9.

Semipalatinsk No. 43.—Only one Semipalatinsk plant produced enough self-fertilized seed to permit the establishment of an inbred line for testing. This plant was rather resistant, and its resistance was carried on in the inbred population, as indicated by the mean, 107.7, of that group. The standard deviation ±30.4 demonstrated considerable variation to exist within the line.

Thus, in all of the varieties and species represented in the first generation of inbreeding, there was a significant tendency for the inbred population from each parent plant to react to black stem in the same manner as the parent had reacted. One exception has been discussed. This tendency in an open-pollinated, heterozygous plant can be explained best on an inheritance hypothesis, for in such a plant, if inheritance were not involved, it is very probable that the observed

correlations of resistance in parent and progenies would not have existed.

Second-generation inbred progenies were obtained from nine of the 18 original plants mentioned above. The others failed to set seed or to produce viable seedlings for various reasons. Comparisons of infection from inoculations made in the greenhouse are shown in table 3. A correlation coefficient of 0.716, significant at the 5-percent level, was obtained between the indices of first- and second-generation progenies, thus further corroborating the evidence secured on first-generation inbreds.

The variation was lower in the S_2 generation than in the S_1. This was true in all but one strain tested and would be expected since homozygosity increases as inbreeding proceeds. The average standard deviation for the S_1 generation was 30.0 as compared with 20.7 in the S_2 generation. Thus inbreeding caused a large reduction in variability. All the variation was not due to the heterozygous nature of a line, as some occurred from the reading method and inoculation technique. This was shown by the variability among the four clonal check plants used with every inoculation. Only on rare occasions would the scores of these clones be identical. In every case, however, the variation between the check plants was small in comparison to the variation within a strain.

TABLE 3.—*Infection means and standard deviations on 9 lines for S_1 and S_2 generations*

Variety	Line	S_1 generation		S_2 generation	
		Mean	Standard deviation	Mean	Standard deviation
Ladak	G	101.8	24.9	81.8	14.5
Kansas Common	B	104.6	37.9	97.7	22.3
Semipalatinsk	J	108.7	30.4	92.1	18.3
Kansas Common	C	114.7	29.0	109.4	17.8
Ladak	H	119.9	32.0	124.3	22.0
Turkistan	N	120.5	32.9	133.2	16.7
Kansas Common Selection	L	124.5	20.1	105.1	27.4
Kansas Common Selection	K	126.2	30.4	116.7	28.0
Hairy Peruvian	D	134.9	32.6	117.0	19.5
Average		117.3	30.0	108.6	20.7

HYBRID STUDIES

The parents in the first cross studied were the highly susceptible inbred plant Hairy Peruvian No. 33 as the male parent and the very resistant *Medicago falcata* No. 39 inbred plant as the female parent. Thirty-three first-generation plants were developed from this cross and tested. The mean of the hybrid group was 93.85, denoting a very high degree of resistance. The standard deviation of ±16.8 indicated low variability in the group. This suggested that the resistance of the *M. falcata* parent was dominant. A reciprocal cross was made in order to check the results. Only four first-generation plants developed for testing. All four were resistant, however, and their mean was 101.7. Thus, insofar as could be judged from these data, resistance also was dominant in the reciprocal cross.

An interesting comparison was noted between the hybrid population and the inbred progeny groups from each of the parents. The mean of the resistance of all the plants in the Hairy Peruvian No. 1 inbred line was 148.8, whereas the mean of the *M. falcata* No. 1 line was 81.61. The mean of the hybrid population from these parents was nearly as low as that of the resistant *M. falcata* No. 39 inbred line, for it was 93.85. Also, the uniformity characteristic of a first-generation hybrid population from a cross involving dominance was observed, for the standard deviation of the group was very low, ±16.8, as compared with the deviations of the inbred groups from the same parents (Hairy Peruvian No. 33, $s = \pm20.1$; *M. falcata* No. 39, $s = \pm20.9$).

The infection data for 10 crosses of open-pollinated plants are shown in table 4. Each cross produced both resistant and susceptible

TABLE 4.—*Means of infection and number of plants in the F_2 generation of the crosses of open-pollinated plants*

Cross No.	Varieties crossed	Number in F_2	Mean
[1] 1	Hairy Peruvian × *Medicago falcata*	18	96
2	Kansas Common × Semipalatinsk	10	131
3	Hairy Peruvian × Kansas Common	104	112
4	*Medicago falcata* × Hairy Peruvian	10	102
5	Kansas Common × Hairy Peruvian	14	85
6	Ladak × Hairy Peruvian	49	104
7	Semipalatinsk × Ladak	126	93
8	Ladak × Hairy Peruvian	24	122
9	_____do_____	66	113
10	Semipalatinsk × Kansas Common	10	102

[1] Includes reciprocal cross.

progenies. The plants were self-pollinated to produce the F_2 populations. The 51 F_1's from the 10 crosses did not differ statistically, perhaps because of the small number of plants available, but there were highly significant differences among the pooled F_2 lines. Since calculations on reciprocal crosses did not show significant differences, it was possible to pool all of the F_2's from a cross thus greatly increasing population size. These F_2 populations exhibited significantly different levels of resistance, which meant that certain of the parent plants were transmitting different reaction tendencies.

A low, nonsignificant correlation between these F_1's and their F_2 progeny was noted in all cases except the one in which 26 F_1 plants were available and tested. The true means of the other F_1 plants probably were not secured since each was represented by only 1 to 7 plants, in contrast to the number of F_2 plants, which ranged from 10 to 126 plants in a population. Undoubtedly the larger numbers in the F_2 generation gave a better estimate of a cross than the mean of the F_1's.

Several crosses and backcrosses among inbred lines were made in the summer of 1941. Eleven had progenies of 11 or more. In table 5 the 11 crosses are listed and infection indices of the F_1 plants are given. Analysis of variance showed that highly significant differences occurred among the crosses of the inbred plants. This was not true of the crosses among the open-pollinated plants reported above.

TABLE 5.—*Means of infection for crosses of inbreds and backcrosses*

Parent varieties	Lines crossed	Number of plants in cross	Mean of infection
(Semipalatinsk × Ladak) × Ladak	7– × F	32	80.7
Kansas Common	C3 × C4	36	86.7
Do	B2 × C3	11	89.6
Do	B8 × C5	14	94.2
Kansas Common × Hairy Peruvian	C3 × D2	11	96.8
Kansas Common	B8 × C4	15	96.9
Do	B2 × B8	40	99.4
Do	C4 × C5	31	103.0
(Ladak × Hairy Peruvian) × Hairy Peruvian	6– × D	31	105.8
(Hairy Peruvian × Kansas Common) × Kansas Common	3– × B	11	111.3
Hairy Peruvian × (Kansas Common × Hairy Peruvian)	D × 5–	12	112.8

The larger numbers present in the crosses of S_1's and backcrosses, the more nearly homozygous constitution of the parents, and the closer selection of parents probably accounted for these different results rather than inbreeding itself. The F_2 population from the crosses among inbred plants was not tested.

EFFECT OF INBREEDING AND SELECTION IN INBRED LINES

The distribution of plant indices of the first and second inbred generations of a resistant Kansas Common plant, B, was studied. The mean of the S_1 generation was 102 with a standard deviation of 37.9. The average score of the original P_1 plant B was 100, which is the result from approximately 10 inoculations, as several cuttings were available. Thus, this plant transmitted resistance to its offspring approximately at its own reaction level. The plant B2, a selection
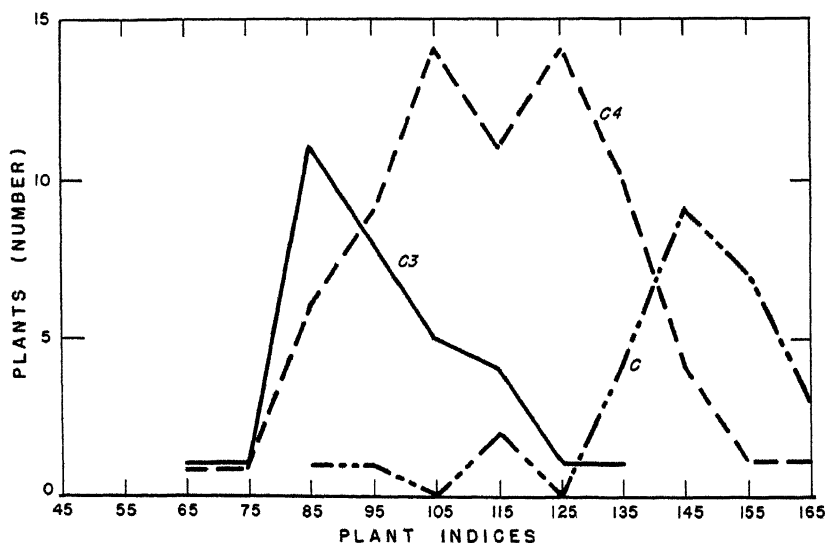

FIGURE 2.—Black stem indices for the first and second generation inbred progenies from the Kansas Common variety of alfalfa. Line C represents the S_1 generation and lines C3 and C4 the S_2 generation. Significant increases in resistance were obtained.

from the selfed progeny of B, scored an average of 93.   Selfed progeny of B2 had a mean of 95 with a standard deviation of 21.4.   Thus B2 transmitted similarly to B.   The progenies of B and B2 did not differ statistically.

Figure 2 shows the distribution of plant indices of the S_1 and S_2 generations of a susceptible Kansas Common plant, C.   It is readily seen from the graph that this plant produced mostly susceptible offspring, as only 2 plants scored less than 100.   The mean of the 27 selfed progenies was 147 with a standard deviation of 29.0.   Plant C3 had an average score of 110, and the average of its 32 selfed progenies was 96.1 with a standard deviation of 14.1.   Plant C4, a sister of C3, averaged 116, and its 72 selfed progenies averaged 115 with a standard deviation of 19.6.   Analysis of variance showed that all 3 progenies differed from each other significantly.   Thus selection raised the resistance of this line considerably above the original level.

Figure 3 shows the distribution of the S_1 and S_2 generation plant indices for a selection from the Hairy Peruvian variety.   Its selfed progeny averaged 140, having a standard deviation of 32.6.   D2, a
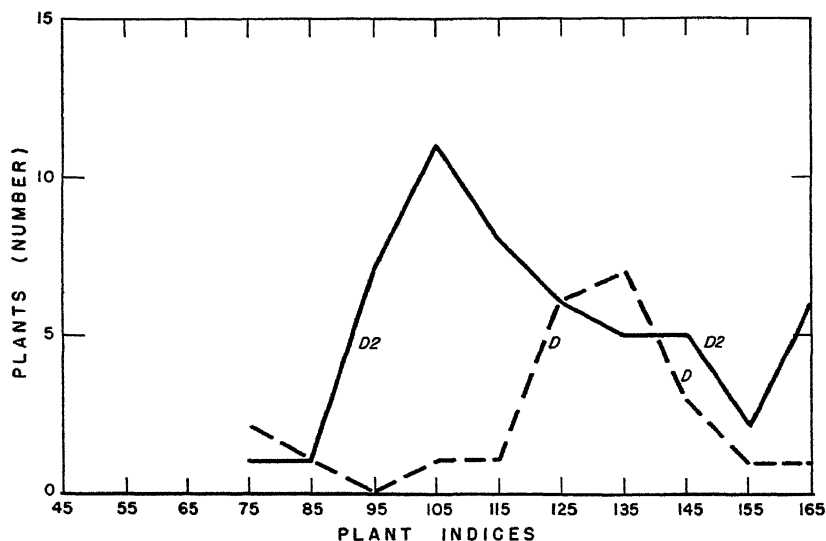

FIGURE 3.—Black stem indices for the S_1 and S_2 generations of Hairy Peruvian alfalfa.   Line D represents the first inbred generation and line D2 the progeny from a resistant selection.

selection from the S_1 progeny scored 117 and the mean of its progeny was 117.   Analysis of variance showed that the progeny of D2 averaged significantly more resistant than the progeny of D.

The plant indices for the S_1 and for the unselected S_2 generations from a Ladak plant, H, were studied.   The S_2 generation was an unselected population, as a number of S_1's were selfed and the seed mixed.   The mean of the S_1 generation was 121 with a standard deviation of 32.   The mean of the S_2 generation was 131 with a standard deviation of 23.1.   The difference between the two means was not significant.   Thus in this case there was no selection and the

S_2 generation was not quite as resistant as the S_1, although the difference was not significant.

These four cases illustrate the value of selecting for resistance to black stem in an inbreeding program on heterozygous material. In all cases the selection of resistant plants in the S_1 generation tended to produce more resistant plants in the S_2 generation than in the S_1, while in the case of an unselected population there was no change in resistance in the S_2 generation as compared with the S_1.

<center>EFFECT OF SELECTION IN HYBRID LINES</center>

Figure 4 shows the distribution of plant indices of two F_2 lines, 3 and 7, derived from crossing open-pollinated plants. Line 3 resulted from crossing a susceptible Hairy Peruvian with a resistant Kansas Common. Line 7 was the cross of a resistant Semipalatinsk by a resistant Ladak. Crosses of two susceptible plants, Ladak by Hairy Peruvian, gave about the same distribution as shown by line 3. The
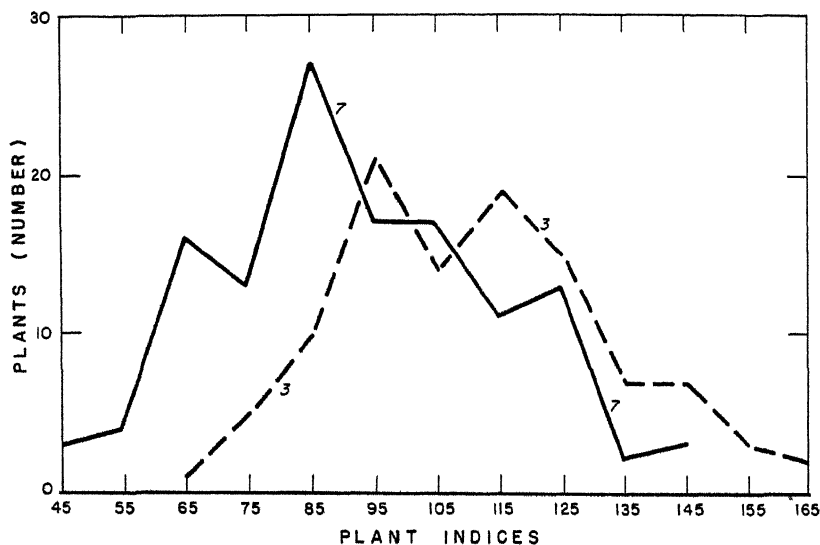

FIGURE 4.—Black stem indices for F_2 plants from crosses of open-pollinated plants. Line 3 represents a cross of susceptible Hairy Peruvian by a resistant Kansas Common and line 7 a cross of resistant Semipalatinsk by resistant Ladak.

means of the F_1's of the three crosses did not differ significantly, but those of the F_2's did. Line 7 was significantly more resistant than the other lines. Thus the cross involving two resistant plants produced more resistant offspring than either of the other two crosses, one of which had one susceptible parent and the other two susceptible parents.

These results corroborate those from crosses of the inbreds. The progeny carried a high degree of resistance if the parents were resistant, and proved susceptible if the parents were susceptible.

SELECTION OF HIGHLY RESISTANT PLANTS

One plant from one of the Kansas Common wilt-resistant selections proved to be highly resistant to black stem. On three successive inoculations it scored 43, 41, and 56 as compared with an average of 82 for the most resistant line included in the same tests. On one inoculation in the fall of 1942 this selection scored 69. A comparison of this plant with Hairy Peruvian is shown in figure 5. Another plant from the F_2 generation of a Semipalatinsk by Ladak cross averaged 48 for two inoculations.
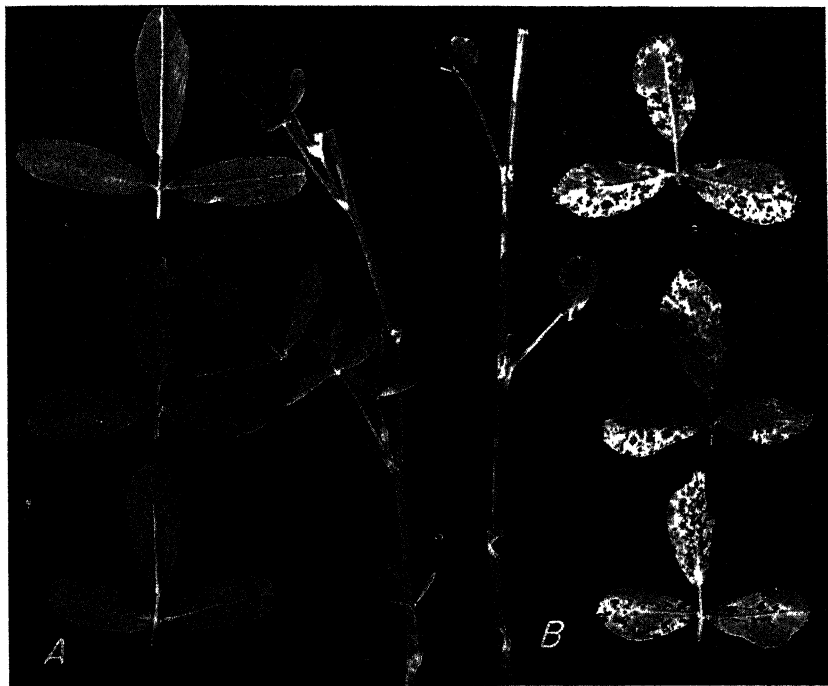

FIGURE 5.—An illustration of extreme resistance to black stem in a selection of Kansas Common alfalfa (*A*), and of susceptibility in a plant of Hairy Peruvian (*B*).

PREDICTION OF THE REACTION OF THE F₁ GENERATION PLANTS

It is generally accepted that the average of the selfed progeny from a plant is a better index of a plant's transmitting ability than the actual score of that plant. For this reason the means of the selfed progeny of each parent in the crosses listed in table 5 were averaged to obtain an expected F_1 index of infection. The correlation of the means of the F_1's and the expected indices was significant at the 5-percent level. This indicated that the F_1's were intermediate between the parental transmitting abilities as measured by the reaction of inbred progeny from the parent plants.

DISCUSSION

These studies showed that inheritance of resistance to black stem was definite but not simple. Intergrading classes of resistance were observed in all populations. Immunity was not observed in any plants. Much variability was found to exist within open-pollinated varieties, among hybrids, and in the selfed progeny from one-generation inbreds. Slight deviations in laboratory conditions resulted in marked differences in infection.

The numerous sources of variation made extensive use of statistical analysis mandatory if a clear view of the results was to be obtained and positive evidence of inheritance revealed. The fact of inheritance of resistance was shown in tests of varieties, various kinds of selections, and in the progeny of hybrid combinations. Even the reactions of the F_1's from crosses of inbreds and backcrosses could be predicted by calculating indices from the reaction of the selfed progeny of the parents. Complete dominance of resistance or susceptibility was lacking except in one case, when dominance of resistance was noted. If a large number of favors and different mechanisms were involved in the inheritance of resistance, then resistance might be both dominant for some genes and recessive for others.

The exact nature of the mild type of resistance observed in these studies is not understood, but it must be either morphological or physiological. In respect to the former, it should be noted that a glossy, hairless leaf surface common in Ladak made thorough wetting of the leaves more difficult than in Hairy Peruvian where the leaf surface is covered with epidermal hairs. This would suggest an escape mechanism. In an effort to discover a physiological factor, an experiment was conducted to test the cell sap extracted from resistant and susceptible plants. The respective saps were sterilized in Erlenmeyer flasks; then the fungus was transferred to the sap and allowed to grow. The resultant mycelial pad weights were compared. There was no difference. These results, while negative, do not prove that living protoplasms may not have differential responses to the fungus.

The question of physiologic races of the fungus was investigated. Diverse collections of the fungus were isolated which showed consistently different growth characteristics on artificial media in the laboratory. Acidified oatmeal agar proved to be the most effective medium for differentiating the collections. Single-spore isolates within a collection gave little or no variation in growth characteristics. Tests of these collections on clonally propagated alfalfa plants showed that strain No. 7 was significantly more virulent than either a mixture of the eight strains or any of the other seven strains individually. This virulence was not correlated with colony growth. The index of infection was 138 for strain No. 7 and 119 for the mixture of all eight strains. Lowest infection was 99 for strain No. 1. Thus strain differences were established. However, in the tests reported here it is considered that this was not an important source of variability.

Resistant selections were obtained among inbred plants derived from certain open-pollinated varieties and hybrid populations and as inbreeding proceeded, variability of reaction within a line declined. Paralleling this means of isolating resistant plants was the success achieved by selecting for resistance without resorting to inbreeding.

Since most of this investigation involved inbreeding, equal numbers of inbred and open-pollinated plants were not studied.   In spite of this, there was evidence, as already pointed out, that the highest levels of resistance achieved from selecting among inbreds were matched by selections in open-pollinated stocks.   Self-sterility, low vigor of inbred lines, and the labor involved reduces the value of inbreeding in an alfalfa improvement program.   It would seem easier to secure natural crosses in a polycross nursery composed of selected plants and, by careful selection among the progeny thus produced, raise the level of resistance.

Several other leaf spot diseases of alfalfa are important and may be confused with black stem both in the symptoms of the disease and the damage done to the plant.   Hence in a breeding program it is important that resistance to all similar diseases be observed.   Greenhouse testing with pure cultures of the fungus could be used to advantage in making specific tests for resistance.

SUMMARY

This paper presents a study of the reaction of alfalfa varieties and strains to the black stem disease caused by *Ascochyta imperfecta* Peck.   The fungus affects the stems and leaves of the plants, causing reduced yield and quality of the crop.

Different varieties of alfalfa showed different levels of infection in the field and in the greenhouse.

Inbred progeny from resistant and susceptible plant selections showed inter- and intravarietal differences.

There was a significant correlation of resistance between S_1 and S_2 lines from five varieties of alfalfa.   Variability was lowest in the S_2 generation.

Crosses among open-pollinated plants reacted similarly in F_1, perhaps because of the small number of plants available, but significant differences were found among lines in the F_2 generation.

Crosses among inbreds and backcrosses showed significant differences.

The F_1's tended to be intermediate between the parents, although in a few cases a tendency toward dominance of resistance was noted.

Inbreeding followed by selection and hybridization followed by selection in F_2 proved to be valuable in raising resistance levels.

The fact that inheritance conditions the resistance of alfalfa plants to black stem was amply demonstrated, but the factors responsible for the inheritance of resistance were not determined.   One plant characteristic which might possibly influence resistance to black stem was noted; namely, the glossy, hairless leaf surface in Ladak which made thorough wetting of the leaves difficult.

Physiological races of the fungus were observed in a study of the growth characteristics of different collections on artificial media. These races showed significantly different virulence on alfalfa plants.